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Post-packaging irradiation combined with modified atmosphere packaging for control of bacterial pathogens on meat products

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Post-packaging irradiation combined with modified atmosphere packaging for control of bacterial pathogens on meat products

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ABSTRACT

Foodborne illnesses caused by consumption of contaminated meat and poultry products with *Escherichia coli O157:H7*, *Listeria monocytogenes*, *Salmonella* or *Campylobacter* have been major concerns in the U.S. Therefore, control of these pathogens by irradiation combined with modified atmosphere packaging (MAP) was investigated. Ground beef patties were inoculated with *E. coli O157:H7*, frankfurters or pre-cooked pork chops were inoculated with *L. monocytogenes*, and fresh chicken breasts were inoculated with *Salmonella enterica* Typhimurium, or *Campylobacter jejuni*. Packaging in vacuum or high CO_2 MAP (99.5% CO_2/ 0.5% CO for beef patties and chicken breasts; and vacuum or 100% CO_2 for frankfurters or pork chops) was used for packaging these products. Products were treated with electron-beam irradiation at refrigerated temperature with target doses of 0 (control), 0.5, 1.0 and 1.5 kGy for beef patties and chicken breasts inoculated with *Salmonella*, 0, 1.0, 1.5 and 2.0 kGy for frankfurters and pork chops, and 0, 0.25, 0.5 and 0.75 kGy for chicken breasts inoculated with *Campylobacter.* Packaging methods did not affect radiation sensitivities of the pathogens. Radiation D_{10}-values, each in vacuum and high CO_2 MAP, respectively, were 0.47 ± 0.02 kGy and 0.50 ± 0.02 kGy for *E. coli O157:H7* on beef patties; 0.66 ± 0.03 kGy and 0.70 ± 0.05 kGy for *L. monocytogenes* on frankfurters, and 0.60 ± 0.02 kGy and 0.57 ± 0.02 kGy for this pathogen on pork chops; 0.55 ± 0.03 kGy and 0.54 ± 0.03 kGy for *Salmonella* on chicken breasts, and 0.31 ± 0.01 kGy and 0.29 ± 0.03 kGy for *Campylobacter* on chicken breasts. Although there was no increase in numbers, *E. coli O157:H7*, *Salmonella* and *Campylobacter* survived in both vacuum and MAP during post-irradiation storage. The growth of *L. monocytogenes* was inhibited in high CO_2

MAP for 12 weeks compared to 7-9 weeks in vacuum. CO in MAP retained red ground beef color during irradiation. Sour-like aroma was detected in the products from high CO_2 MAP, while irradiated off-odor was observed in all irradiated meat.

CHAPTER 1—GENERAL REVIEW OF LITERATURE

INTRODUCTION

Foodborne illnesses caused by contaminated meat products have been a major concern of the meat industry in the U.S. The increasing size of animal production facilities, the gradual change to centralized meat processing with larger product volumes, and the changes in eating habits of consumers (from eating at home to eating in the restaurants and eating more fast food) have created more difficulties and challenges for control of foodborne pathogens (Miller and others 1998; Juska and others 2003). Foodborne illnesses caused by consumption of meat and poultry products contaminated with *Eschrichia coli O157:H7*, *Listeria monocytogenes*, *Salmonella* and *Campylobacter* are among the most prominent concerns for public health (Smith 1998; Mead and others 1999; Dewaal and others 2006). Even though outbreaks caused by these microorganisms declined in 2004 from the baseline in 1996-1998 (CDC 2005; USDA-FSIS 2005a), development of intervention strategies to further improve control of these four pathogens in meat products is still considered a high priority for the U.S.(USDA-FSIS 2003b).

Escherichia coli O157:H7

This bacterium is also called enterohemorrhagic *E. coli* (EHEC), one of five virulence groups of *E. coli* that have been recognized. *E. coli O157:H7* is gram-negative rod-shaped facultative mesophile. The optimal growth temperature was reported to be 35 °C to 37 °C, with a minimum growth temperature of 8 °C to 10 °C (Rajkowski and Marmer 1995). This pathogen produces Shiga toxin(s), which is responsible for the acute

bloody diarrhea and hemolytic uremic syndrome (HUS) which often results in kidney failure. Children under 5 years old and the elderly are most vulnerable to this disease (CDC 2007a). It was estimated that this foodborne pathogen has caused 73,000 cases of infection and 61 deaths in the United States each year (Mead and others 1999). Cattle have been identified as major carriers for *E. coli O157:H7*, which exists in bovine intestines as a normal flora. This microorganism spreads through seasonal shedding, contamination of hides on live animals, animal transportation, holding and harvest (Vanselow and others 2005). Because this pathogen is able to adapt to multiple stressors, such as acid resistance, sanitation detergents and low temperature, meat processing plants have been facing tremendous challenges in controlling this organism on beef products, especially ground beef (Farrell and others 1998; Rivera-Betancourt and others 2004; Edwards and Fung 2006). Although this pathogen is heat sensitive and can be destroyed by cooking meat products to the internal temperature of 72 °C, undercooked hamburgers or cross contamination of ready-to-eat foods with raw meat have often been related to outbreaks (Miller and others 1998). Due to the low infectious dose (1-200 cells), a high mortality rate among infected young children and elderly (2-10%) and the severe complication of HUS, the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) issued a zero tolerance policy for *E. coli O157:H7* in raw ground beef in 1994 (Law 2000; USDA-FSIS 1999a). Since then, along with recalls of possible contaminated meat products (primarily ground beef), intensive intervention strategies have been launched for control of this foodborne pathogen. As a result, according to the the Center of Disease Control and Prevention (CDC)- Morbidity and Mortality Weekly Report (CDC, 2005), foodborne illnesses caused by *E. coli O157:H7* declined 42 % in 2004, compared with the cases reported in 1996-1998. The reported

cases have met the National Healthy People 2010 goal (U.S. Department of Health and Human Services 2000; USDA-FSIS 2005c), which was 1 infectious case per 100,000 persons.

Listeria monocytogenes

L. monocytogenes is gram-positive, rod-shaped, facultative psychrotroph. The optimal growth temperature was reported to be 35 °C, with a minimum growth temperature of 0.5 °C to 3.0 °C, and a maximum growth temperature of 45 °C (Jay and others 2005). *L. monocytogenes* can be found in the soil, water and many other areas in food processing environments, such as walls, drains, belts, conveyers and machine surfaces (Beresford and others 2001; Chasseignaux and others 2002; Tompkin 2002; Lunden and others 2003). Raw vegetables and meats can be easily contaminated with this organism (Thevenot and others 2006). Because this pathogen can grow at refrigeration temperature, many ready-to-eat (RTE) foods can be contaminated or cross contaminated in chillers or home refrigerators where raw foods and RTE foods are normally stored together (Glass and Doyle 1989; Cates and others 2006; Jackson and others 2007). Although this microorganism can be inactivated through cooking of raw meat, RTE meat is normally consumed without re-heating. It was estimated that 2,500 persons are infected with listeriosis each year in the United States and 500 die, with 90% of the cases caused by consumption of ready-to-eat food products contaminated with *L. monocytogenes* (Mead and others 1999; CDC 2007b). Pregnant women and their fetus or newborns, people with compromised immune system such as those with cancer, diabetes, kidney disease or AIDS, and the elderly are most vulnerable to listeriosis. A major challenge for the meat industry to gain control of this foodborne pathogen in ready-to-eat meats is that

L. monocytogenes can withstand many harsh treatments such as heat, freezing, drying, high salt concentrations and sanitation treatments that are normally used to preserve food products or to clean the food processing environments (Lou and Yousef, 1996, 1997; Zhu and others 2004). Once the biofilm of this organism is formed on food contact surfaces, it becomes very difficult to eliminate the pathogen by ordinary sanitation procedures; therefore, RTE meat products can be cross-contaminated during post-cooking processes, such as peeling and slicing, or post-process packaging, and then the pathogen can continue to grow on the product at refrigeration temperature to reach infective levels (Jessen and Lammert 2003; Somers and Wong 2004; Lin and others 2006; Wilks and others 2006; Vorst and others 2006a, 2006b). Therefore, deli meats and non-reheated frankfurters are listed in a high risk category for causing listeriosis in the U.S. (FDA, USDA and CDC 2001). According to the federal law, ready-to-eat meat and poultry products contaminated with *L. monocytogenes* are adulterated (USDA-FSIS 2007). After the multi-state outbreaks of listeriosis caused by unheated frankfurters and deli turkey meat in 1998 and 2002, the FSIS issued a final rule in 2003 requiring processed meat companies to develop three scientifically validated alternative programs for the control of *L. monocytogenes* in RTE meat and poultry products (USDA-FSIS 2003a; Gottlieb and others, 2006; Mead and others 2006). The three alternatives are: (1) apply both post-packaging lethal treatment and a growth inhibitor of *Listeria* in RTE meats along with sanitation measures, (2) apply either post-packaging lethal treatment or a growth inhibitor along with sanitation, (3) apply Good Manufacture Practices (GMP) and sanitation. The final rule requires that *L. monocytogenes* be considered as a hazard and included in Hazard Analysis and Critical Control (HACCP) plans of processed meat establishments. The USDA Food Safety and Inspection Service increased the inspection and sampling in

establishments producing RTE meat to provide incentives for the industry to increase the testing of this organism and incorporate other preventive measures to control or eliminate this food-borne pathogen (USDA-FSIS, 2003a). The companies that apply only the third alternative intervention are under most frequent scrutiny by FSIS. Many studies have been done on control of *L. monocytogenes* on RTE meat products, and several intervention measures have been applied in the meat industry. As a result of this endeavor, it has been estimated that infections caused by the pathogen decreased 40% from 1996 to 2004 (2.7 cases per million population), however, a sharp increase in the number of infections occurred in 2003, indicating that further efforts are still needed for the improvement of control measures (CDC 2005). According to a risk assessment panel, among all the intervention strategies that have been applied for reducing listeriosis, improved control of the growth of *L. monocytogenes* to prevent an infective dose on RTE food products is a most effective way to reduce this foodborne disease (Walls 2005).

Salmonella

This organism is a gram-negative, rod-shaped, facultative mesophile, and belongs to the family of *Enterobacteriaceae,* genus *Salmonella*. The optimal growth temperature is 37 °C, with a minimum growth temperature of 5.3 °C to 6.2 °C, and a maximum temperature of 45 °C. There are approximately 2000 serotypes that cause human disease (Jay and others 2005). It has been estimated that 1.4 million cases of salmonellosis occur annually in the United States, with 2% fatalities (CDC 2007c). Among those cases, 50% of the illnesses are caused by *Salmonella enterica* Typhimurium and *Salmonella enterica* Enteritidis. The control of *Salmonella* infections originating from meat products requires a farm-to-table multistage system similar to control of infections caused by *E. coli*

O157:H7 and *Campylobacter*. The pathways for *Salmonella* to spread include seasonal shedding in farm animals and birds (chickens and turkeys), transportation of animals and birds to processing companies, fecal contamination of animal and bird carcasses during processing, contamination of processing equipment, and further contamination or cross-contamination of meat and poultry products during wholesale or retail distribution (Zhao and others 2001; Fries 2002; Hurd and others 2002, 2005; Smith and others 2005a; Rodriguez and others 2006). In meat products, *Salmonella* can be destroyed by cooking the meat to the internal temperature of 72 °C (Jay and others 2005). Consumption of undercooked meat products, cross-contamination of RTE food with raw meat, poultry or eggs, and RTE food handled with poor personal hygiene are typical causes of foodborne salmonellosis (Kusumaningrum and others 2004; McLaughlin and other 2005; CDC 2007c). Along with the implementation of Pathogen Reduction and HACCP system in the meat industry, FSIS has been utilizing a sampling and testing program for *Salmonella* in meat and poultry establishments to enforce control of this pathogen in raw meat and poultry products (USDA-FSIS 2006). The meat and poultry industry has been applying various control measures to reduce the contamination (Mead 2004). It was reported by CDC (2005) that *Salmonella* infections decreased 8% in 2004 compared with the baseline of 1996-1998; however, only the infections caused by *Salmonella* Typhimurium decreased significantly; the infection rate caused by *Salmonella* Enteritidis did not change. Another concern has been for salmonellosis cases caused by a multidrug-resistant strain of *Salmonella* Newport which increased 41% in 2004 (USDA-FSIS 2006; Verma and others 2006). The FSIS also reported that although the positive samples of *Salmonella* collected from raw meat and poultry decreased from 10.65% in 1998 to 3.8% in 2003, the rate of positive sample in broilers increased from 11.5% in 2002 to 12.8% in

2003 (USDA-FSIS 2005b; Altekruse and others 2006; Naugle and others 2006) suggesting that improved control of *Salmonella* in poultry products should be considered an important priority.

Campylobacter jejuni

This pathogen is a gram-negative, microaerophilic, spiral rod-shaped microorganism. The optimal growth temperature was reported to be 42 °C, with a minimum growth temperature of 30 °C to 31 °C (Hazeleger and other 1998; Duffy and Dykes 2006). *Campylobacter* colonizes especially well in bird (chickens, turkeys and others) intestines as normal microflora (Jay and others 2005; Johansen and others 2006). *Campylobacter* can also be found in the intestinal tracts of cattle, swine and other animals, and in untreated water (Karenlampi and others 2007). This pathogen is one of the most frequently reported pathogens causing human foodborne diarrhea (20 cases per 100,000 population diagnosed in the United States). Most of human campylobacteriosis cases are caused by *Campylobacter jejuni*, with about 1% caused by other species (CDC 2007d). According to the estimation by CDC, 2.4 million persons become ill each year in the U.S. from consumption of foods contaminated with *Campylobacter*, including unpasteurized milk, undercooked meat and poultry, and RTE foods cross contaminated with raw meat and poultry (Mead and others 1999; Kusumaningrum and others 2004; Luber and others 2005). In the meat and poultry industry, controlling infections of *Campylobacter* is another farm-to-table effort involving animal producers and meat and poultry processors (Keener and others 2004; McCrea and others 2006; Wagenaar and others 2006). The pathway for the pathogen to spread is similar to *Salmonella*; however, some studies have suggested that there might be more complex factors and variables associated with the

spreading of *Campylobacter* from chickens to humans (Wilson 2002; Stern and Robach, 2003; Mead 2004; Son and others 2007). In recent years, *Campylobacter* counts have been found to be higher on raw poultry products than on other meat types, although cases of campylobacteriosis declined 31 % in 2004 relative to 1996-1998 (CDC 2005; Stern and Pretanik 2006). Studies have shown that *Campylobacter* preferred to grow in the environment of live chickens and chicken meat, and normal decontamination methods have not been sufficient for elimination of the pathogen from poultry products (Ingmer and others 2004; Dhillon and others 2006; Wingstrand and others 2006). Research also showed that, although *C. jejuni* is considered a thermophilic microaerophile with optimal growth temperature of 42 °C (close to chicken body temperature), this pathogen can survive many stressors, including refrigeration, freezing and modified atmosphere packaging (Beuchat 1985; Grigoriadis and others 1997; Moore and others 2002; Bhaduri and Cottrell 2004; Murphy and others 2006; Ritz and others 2007). Furthermore, studies have shown that when *C. jejuni* infects humans, the human body temperature (37 °C) encouraged the pathogen to express greater chemotaxis, suggesting that this pathogen is more virulent to humans than to birds (Khanna and other 2006). Similar to *Salmonella*, *Campylobacter* in poultry has also developed antibiotic resistance which poses a special risk for humans (Luangtongkum and others 2006). The FSIS called on the poultry industry to make more efforts to control the prevalence of *Campylobacter* along with control of *Salmonella* on poultry products. The Agency has suggested that intervention strategies include development of reliable and consistent methodologies for the detection of *Campylobacter* in live birds and poultry products, implementation of new validated control measures, and collection of the most complete data available on the behavior of this microorganism on farms and in processing plants (USDA-FSIS 2005b).

For control of foodborne pathogens which spread through colonization in animals and subsequent shedding, such as *E. coli O157:H7*, *Salmonella* and *Campylobacter*, on-farm controls such as water treatment with chlorine, animal hygiene and on-farm sanitation have been taking place for several decades (Hussein and Sakuma 2005). Extensive on-going research has also been heavily focused on control of these pathogens in live animals or birds in many ways including various feed additives (Van Immerseel and others 2002), competitive exclusion with harmless anaerobic bacteria (Schneitz 2005), bacteriophage therapy (Carrillo and others 2005), feed withdrawal (Reid and others 2002) and others.

In meat processing companies, in addition to the ante-mortem and post-mortem inspection conducted by FSIS, plant sanitation and carcass decontamination also play very important roles in controlling foodborne pathogens on final products. Extensive studies have been done on physical and chemical methods for removing pathogenic bacteria from animal and bird carcasses, including steam vacuuming, trimming, pre- chill hot water plus organic acid (or other antimicrobials) washing, post-chill acids prays and others (Juneja 2004). Although methods for food preservation, such as heating, drying, salting and fermentation date back to ancient history, these technologies are still applied in modern times for control of the growth of food spoilage bacteria and foodborne pathogens by changing intrinsic or extrinsic environments of microorganisms (Jay and other 2005). While those conventional methods are still commonly used in the meat industry, relatively new technologies, such as irradiation, high hydrostatic pressure, modified atmosphere packaging and bio-protection, have been also proved to be effective for pathogen intervention purposes (Juneja 2004). Although these control measures are categorized as thermal and non-thermal treatments, the interactive effects of these

technologies on the behavior of microorganisms has become better understood; therefore, combinations of interventions (hurdle technology) have been developed (or are developing) not only for elimination of bacteria, but also for preservation of the quality of food products (Leistner 2000; Dincer and Baysal 2004; Manas and Pagan 2005).

The objective of this review is to summarize some recent control measures that have been used for *E. coli O157:H7*, *L. monocytogenes*, *Salmonella* and *Campylobacter* in meat and poultry products. Hurdle technologies are presented in this review, and modified atmosphere packaging (MAP) and food irradiation are discussed in detail because these are the hurdles evaluated as part of the objectives of the present study.

THERMAL TREATMENTS AND HURDLES

Thermal treatment is still the most common intervention method for meat products. There are science-based compliance guidelines written in the federal law (9 CFR 301, 303, 318.17, 318.150, 417) regarding the temperatures and times for inactivation of foodborne pathogens in RTE meat and poultry products (USDA-FSIS 2005d). Research must be done to validate processing information for the meat industry or food services, so that a proper temperature and time can be used as a critical control point (in HACCP) for a specific RTE product (USDA-FSIS 2003b, USDA-FSIS 2005c; O'Bryan and others 2006). In recent years, many studies have been focused on the heat treatments necessary for cooking raw meat and poultry to achieve safety, or for eliminating foodborne pathogens (especially *L. monocytogenes*) from post-thermal processed or packaged RTE meat and poultry products.

Cooking raw meat and poultry products

The temperature and time for inactivation of *E. coli O157:H7* in ground meat and poultry were validated by Juneja and Marmer (1999). According to the study conducted by these authors, cooking to the internal temperature of 65 °C for at least 7.25 min was adequate to reduce 5 log of *E. coli O157:H7* in 90% lean ground beef. The same temperature and time can also be used to eliminate the pathogen in ground chicken, turkey, lamb and pork. Murphy and others (2002a) validated the D-values (decimal reduction time at a specific temperature) and z-values (temperature difference required for inactivate 1 log of bacteria) at 55 °C and 70 °C for the reduction of *Listeria innocua* and *Salmonella* serotypes in several commercial meat products, including chicken patties, chicken tenders, frankfurters, beef patties and patties made with beef and turkey. Murphy and others (2004a, 2004b, 2004c, 2004d) also validated the heat resistance of *E. coli O157:H7*, *Salmonella* and *L. monocytogenes* in ground beef, ground pork, ground turkey, ground chicken thigh/leg meat and chicken skin, and formulated ground beef/turkey links. The D-values and z-values of each pathogen were different, and were product dependent. *E. coli O157:H7* was less heat resistant than *Salmonella* and *L. monocytogenes* at 55 °C or 70 °C. Therefore, these authors suggested that if a thermal process is designed for inactivation of *Salmonella* and *Listeria*, it will inactivate *E. coli O157:H7* as well. In these studies, when the ground beef and turkey patties were cooked to internal temperature of 70 °C, and beef/turkey links were cooked to internal 71 °C, a 7 log reduction of each of these pathogens was achieved. Sallami and others (2006) reported that the cooking-cooling procedures used in the meat industry to produce bologna (internal temperature of 73 °C before showering with cold water) was sufficient for a 5 log reduction of *L. monocytogenes* and *Salmonella* Typhimurium. Murphy and others

(2004e) predicted that the procedure used to process frankfurters plus post-packaging pasteurization treatment was more than sufficient to achieve a 7 log reduction of *L. monocytogenes* in the product.

Many studies have suggested that cooking methods in addition to product temperature also play an important role for inactivation of foodborne pathogens in meat and poultry products. The information is especially critical for food services and cooking at home. Ou and Mittal (2007) predicted (with mathematical heat and mass transfer models) that if frozen hamburger patties were pan-fried on a grill at 140 °C or 160 °C, increasing the frequency of overturning patties (flipping) can reach an internal temperature of 71 °C faster, and 12 log cfu /g of *E. coli O157:H7* can be inactivated at this internal temperature; however, for reduction 12 log of *Salmonella* and *Listeria* at the same internal temperature, longer holding time is needed. Higher pan temperature (180 °C) can decrease the process time; however, this will increase the moisture and fat loss from the product. Rhee and others (2003) also reported that using a double-sided grill (cooking the top and bottom of a patty at the same time) or flipping patties frequently (every 30 seconds) increased internal temperature to 71.1 °C faster, and 7 log of *E. coli O157:H7* in ground beef patties were reduced by using these two methods to reach the internal temperature. However, D'Sa and others (2000) pointed out that a rapid increase in the internal temperature of beef patties cooked with the double-sided grill resulted in springy texture; when comparing beef patties cooked to internal temperature of 60 °C and 68 °C, the higher temperature produced harder and more chewy beef patties. Whyte and others (2006) reported that when pan frying was used to cook chicken livers, achieving internal temperature of 70 °C-80 °C for 2-3 min was necessary for inactivation of 4 log *Campylobacter* spp.

Some thermal treatments have been introduced to eliminate pathogenic bacteria from raw meat and poultry product without cooking the products. Morgan and others (1996) used high temperature (145 °C) and short time (25 milliseconds) treatment to pasteurize the surface of fresh chicken meat and observed a 4 log reduction of *Listeria inocua* with this method. Logue and others (2005) reported that treatment with steam for 10 seconds reduced *E. coli O157:H7* by 1.5 log /cm^2 on beef sirloin slices before vacuum packaging. McCann and others (2006) used dry air with heat (90 °C or 100 °C) to reduce *E. coli O157:H7* and *Salmonella* Typhimurium on beef slices and observed that 4-6 log of these pathogens were reduced with this method; however, the dry air had a negative effect on beef color. *E. coli O157:H7* and *Salmonella* may be to be able to survive fermentation during the production of pepperoni, salami and other fermented meat products (Smith and others 1975a, 1975b; Duffy and others 2002). Heating these products to the internal temperature of 60 °C immediately after the fermentation is necessary to ensure safety.

Heat treatment for pre-cooked meat and poultry products

The contamination with pathogenic bacteria, such as *L. monocytogenes* or *Salmonella*, on RTE meat normally occurs on the surface of products during post-cook or pre-packaging processes. Therefore, surface decontamination methods have been developed for inactivation of these microorganisms prior to or immediately after the packaging process (Houben and Eckenhausen 2006). Surface post-packaging pasteurization with high temperature steam (115 °C-138 °C) or immersion in hot water (90 °C- 96 °C) have been commonly used by processed meat companies to achieve 4-5 log reduction of *Listeria* (Cygnarowicz and others 1994; Kozempel and others 1999;

Muriana and others 2002). Grande and Muriana (2003) and Muriana and others (2004) combined radiant heat as pre-packaging treatment with submersion in hot water as post-packaging treatment for inactivation of *L. monocytogenes* in roast beef or deli turkey meat and a 2-4 log reduction of the pathogen was achieved. Murphy and others (2001) reported that it took 40 min in a batch pasteurization to reach an internal temperature of 70 °C in fully-cooked, vacuum- packaged chicken breast strips (454 g /package) when using steam. To inactivate 7 log *Salmonella* Senftenberg and *L. innocua* on the product, 34 min was needed; however, if the same product was immersed in hot water at 88 °C, the 7 log reduction time was dependent on the product package size; in this case, 34 min was needed for 454-gram packages and 20 min for 227-gram packages (Murphy and Berrang 2002a). However, Murphy and Berrang (2002b) also observed that post-packaging pasteurization steam or hot water (88 °C) increased package purge of the chicken breast strips. Murphy and others (2005a) studied pre-packaging pasteurization for inactivation of *L. monocytogenes* on fully cooked bologna with pressurized steam. This method achieved a 2 log reduction of the pathogen with 75-90% less time than ambient steam. These authors suggested that pressurized steam can also kill bacteria hidden in crevices, dents, cuts, folds or other areas of the products that can protect bacteria from exposure to the heat. These authors proposed that this method be integrated into vacuum packaging systems. Murphy and others (2005b) reported that an integrated system with pressurized steam or hot water before vacuum packaging achieved a 3 log reduction of *L. monocytogenes* on fully cooked frankfurters. Microwave heating is another emerging technology under study for post-packaging pasteurization. Huang (2005) reported that a 7 log reduction of *L. monocytogenes* on frankfurters was achieved

by a computerized on-off control mechanism to heat beef frankfurters for 12-15 min in a 600-W microwave oven.

Many studies have shown that combining other hurdles with heat treatment can reduce the heat resistance of foodborne pathogens. Addition of 4% sodium lactate, for example, to beef patties can reduce the heat resistance of *E. coli O157:H7* and increase the reduction rate of the pathogen at normal cooking temperature (Byrne and others 2002b). Similarly, addition of 3% sodium lactate and 0.25% sodium diacetate to injection brine has been shown to increase the sensitivity of *E. coli* in meat products to heat treatment (Wicklund and others 2005). However, Murphy and others (2004f) reported that addition of sodium lactate to ground chicken thigh meat increased the heat resistance of *L. monocytogenes*, however, did not affect the heat sensitivity of *Salmonella*. Although combining heat treatment with addition of organic acids and antimicrobials might not effectively reduce heat resistance of *L. monocytogenes* on RTE meat and poultry products, these compounds were effective for controlling the growth of the pathogen survivors following heat treatment and during subsequent refrigerated storage (Chen and others 2004; McCormick and others 2005; Luchansky and others 2006; Murthy and others 2006).

Many intrinsic and extrinsic factors have been reported to affect heat resistance of foodborne pathogens. Murphy and others (2002b) reported that the thickness of packaging film for cooked chicken breast meat altered the effectiveness of heat for inactivation of *Salmonella* and *L. innocua*. Ingredients used in meat products, such as seasoning, salt, soy protein, onion, kappa-carrageenan and alginate, protected *E. coli O157:H7* and *L. monocytogenes* in beef hamburgers from heat destruction (Harmayani and others 1993 Byrne and other 2002a; Passos and Kuaye 2002). Blackburn and others

(1997) predicted with thermal inactivation models that sodium chloride below 8.5% can protect *E. coli O157:H7* from heat inactivation, and 5-7% sodium chloride were optimal for *Salmonella* Enteritidis to survive heat treatment. Kotrola and Conner (1997) observed that the mixture of additives (8% sodium chloride, 4 % sodium lactate and 0.5 polyphosphate) that is normally used in a RTE meat formulation enhanced the survival of *E. coli O157:H7* following cooking of ground turkey meat. Fat content in ground beef also increased the heat resistance of *E. coli O157:H7*, and freezing before cooking decreased the heat sensitivity of the pathogen (Ahmed and others 1995; Smith and others 2001). Product pH that is less than 5 or greater than 7 can decrease the heat resistance of these pathogens. Adaptation to stressors such as heat shock, ethanol and hydrogen peroxide sanitizers and low pH increased the thermal tolerance of *L. monocytogenes* (Linton and others 1990; Lou and Yousef 1996; Juneja and other 1998). Riordan and others (2000) observed that acid-adapted *E. coli O157:H7* in pepperoni was more heat sensitive than non-acid-adapted cells. The model developed by Juneja and Eblen (1999) indicated that temperature, pH, sodium chloride and sodium pyrophosphate interacted with one another to affect the heat sensitivity of *L. monocytogenes*, therefore, processed meat companies should design a optimal combination of these factors to facilitate the heat destruction of the pathogen.

While many studies have been focused on heat sensitivity of pathogens in meat and poultry products, there have not been many reports that have evaluated quality and sensory properties of those products after specific heat treatments. Houben and Eckenhausen (2006) pointed out that higher temperature and longer holding time are needed for complete inactivation of foodborne pathogens in RTE meat due to the surface imperfections on various products; however, the quality of RTE meats can be affected.

Selby and others (2006) also reported that post-packaging pasteurization affected meat quality, resulting in increased lipid loss and moisture loss, changed meat color and increased lipid oxidation in RTE meat. Therefore, those authors suggested that more studies are needed on meat formulation changes to avoid the negative effects from post-packaging heat treatments.

NON-THERMAL TREATMENTS AND HURDLES

Since the final rule for control of *L. monocytogenes* in RTE meat and poultry products was issued by the Food Safety and Inspection Service (USDA-FSIS 2003a), several antimicrobials that are generally recognized at safe (GRAS) have been added to the formulations of most RTE meat and poultry products in the U.S. (Giese 1994; Islam and others 2002). These compounds have included organic acids and organic acid salts, and natural products such as essential oils. In recent years, studies have also included foodborne or meatborne antagonistic lactic acid bacteria, bacteriocins, and bacteriophages for control of foodborne pathogens in meat and poultry products. To meet consumer preferences for minimal processed meat products, other non-thermal treatments, such as high pressure, modified atmosphere packaging and irradiation, have received a great deal of attention from the research community and the industry (Juneja 2004).

Antimicrobials that are generally recognized as safe (GRAS)

The GRAS compounds that have been studied include lactic acid, acetic acid, citric acid, benzoic acid and their respective salts, medium-chain free fatty acids and bacteriocins such as nisin. While lactic acid, acetic acid and fatty acids are often applied

as carcass washes or for treating raw meat cuts or trimmings, sodium lactate, potassium lactate and sodium diacetate are most commonly used in RTE meat and poultry products.

Treatments for raw meat and poultry products: Ellebraht and other (1999) demonstrated that washing beef trimmings with hot water and 2 % lactic acid significantly reduced *E. coli O157:H7* and *Salmonella*, especially when the treated product was stored at 4 °C. However, this treatment also resulted in darker lean meat color and softer fat. On the other hand, studies conducted by Brackett and others (1994), Conner and others (1997) and Uyttendaele and others (2001) suggested that washing beef trimmings with lactic acid or acetic acid (with or without hot water) was not effective for reduction of *E. coli O157:H7*, and the quality of the product was also negatively affected. Smulders and Greer (1998) suggested that organic acids might not affect meat quality when applied as a carcass wash, however, when directly applied to meat cuts, sensory properties of the resultant meat products may be changed. Lim and Mustapha (2003) used low molecular weight polylactic acids as a dipping solution to control *E. coli O157:H7* on vacuum packaged fresh beef cubes. The organism was reduced by approximately 7 log /cm^2 after storage at 4 °C for 42 days. These authors reported that the pH of the beef cubes decreased to 4.2 immediately after the acid treatment and increased to 5.3 after 1 hour following treatment; during storage, the meat pH was significantly lower for the treated samples than for the untreated product. This study did not include other quality evaluations such as package purge or color change (often related to low pH meat). In a similar study conducted by Mustapha and others (2002), low molecular weight polylactic acid did not show a significantly different effect than lactic acid when used as a dipping solution for fresh beef cubes, and neither acid was bacteriocidal for *E. coli O157:H7* immediately after treatment or after 28 days of storage at 4 °C. Kang and others (2001a,

2001b) used multiple steps involving lactic acid washes, short time hot water washes and hot air to decontaminate beef trimmings. Coliforms and *E. coli* on beef trimmings were significantly reduced immediately after the treatment and during refrigerated storage, however, quality changes of beef trimmings after the multiple step treatment was not reported. Mbandi and others (2004) studied the efficacy of medium-chain free fatty acids for control of *L. monocytogenes* on beef emulsions and frankfurters, and reported that lauric acid (500 ug/g or lauric acid (500 ug /g) plus capric acid (300 ug /g) inhibited the growth of the pathogen for 21 days at refrigeration temperature by reducing the pH of the products; however, sensory properties of these products were not reported. Deumier (2006) used a 1 % lactic acid solution plus vacuum tumbling to decontaminate chicken legs, and reported that *Salmonella* positive samples were significantly reduced by this method in comparison to the non-treated group (4.83% versus 13.15%) without a negative sensory effect. However, in the previous study (Deumier 2004), the author reported that 1 % lactic acid induced yellowness and greenness on chicken skin. Gonzalez-Fandos and Dominguez (2006) also observed that dipping chicken legs in lactic acid solution inhibited the growth of *L. monocytogenes* and other spoilage bacteria during storage time, however, the acid resulted in a pale appearance on the chicken legs. Carroll and others (2007) combined lactate and diacetate with other ingredients for marination of turkey breast. The growth of *L. monocytogenes* on deli meat made from the marinated turkey was delayed for 74 days during refrigerated storage.

Treatments for RTE meat and poultry products: A common practice in the processed meat industry for control of *L. monocytogenes* on RTE meat during storage is to use 2-3% sodium lactate (or potassium lactate) with or without sodium diacetate (0.25%). Lactate, as one of ingredients of RTE meat products, has little negative impact

on quality and sensory properties (Blom and others 1997; Bedie and others 2001; Glass and others 2002; Stekelenburg 2003). The inhibition effect can last 4-12 weeks depending on the products or product formulations. Studies have also shown that control of *L. monocytogenes* on RTE meat products, such as frankfurters and bologna, can be improved by dipping the products in solutions of organic acids and/or the salts. Samelis and others (2001) observed that dipping sliced pork bologna in 2.5 or 5% acetic acid, 5% sodium diacetate or 5% potassium benzoate for 1 min prior to vacuum packaging, controlled growth of the pathogen for 120 days at 4 °C; dipping with 5% potassium sorbate or 5% lactic acid delayed the growth for 50 to 90 days; and dipping with 5 or 10% sodium lactate inhibited the growth for 20 to 35 days. Lu and others (2004) attempted to combine sodium diacetate and potassium benzoate, or sodium lactate, sodium diacetate and potassium benzoate and dipping solutions for control the pathogen on frankfurters. These authors observed that, among all the combinations, 6% sodium diacetate alone was more effective than the other combinations of treatments. Barmpalia and others (2004), however, reported that addition of sodium lactate (1.8%) and sodium diacetate (0.25%) in frankfurters was more effective for control of *L. monocytogenes* when the product was exposed to mild temperature abuse at 10 °C than formulating these compounds into the product, plus dipping the product in the solution oflactic or acetic acid before packaging. These authors (Barmpalia and others 2005) observed a similar listeriostatic effect when these two compounds were included as ingredients in pork bologna. While these additives have been shown to be effective for control of the pathogen, studies have also reported that these compounds reduced the redness of cured meat products (Peirson and others 2003; Lu and others 2005; Carroll 2007). In addition to the antimicrobials mentioned above, Glass and others (2007) showed that a combination

of sodium benzoate (0.05%) and sodium propionate (0.05%) or potassium sorbate in turkey and pork-beef bologna inhibited the growth of *L. monocytogenes* for 6-13 weeks during refrigerated storage. Burnett and other (2005) dispensed a 1% octanoic acid solution from a packaging film (about 0.12 ml /cm^2) and were able to reduce *L. monocytogenes* on the surface of turkey (1.46 log reduction) and ham (3.34 log reduction). A similar method was used by Luchansky and others (2005), whereby, a acidic calcium sulfate and lauric arginate solutions were immobilized in the film of shrink-wrap bags and reduced *L. monocytogenes* on ham surfaces within the first 24 hours at 4 °C, and further inhibited the growth of the pathogen during subsequent refrigerated storage.

Non-meat ingredients

Processed meat ingredients such as sodium nitrite, orthophosphate and liquid smoke have significant antimicrobial properties (Giese 1994; Capita and other 2001; Estrada-Munoz and others 1998). Sodium nitrite acts not only as a curing agent for enhancing the color and flavor of cured meat products, but also as potent antimicrobial agent to suppress the growth of *Clostridium botulinum* (Cammack and others 1999) and *L. monocytogenes* (Whiting and Masana 1994). Ngutter and Donnelly (2003) observed that 100 and 200 ppm of sodium nitrite used in the formulation of frankfurters injured more than 98 % *L. monocytogenes* inoculated on the products. Ingham and others (2006) reported that dry-curing was an important step in the processing (dry curing, rinsing, pressing and drying) for control *Salmonella*, *E. coli O157:H7* and *L. monocytogenes* in a dry-cured meat product (basturma). Birzele and others (2005) observed that sodium nitrite inhibited the growth of *L. monocytogenes* on fresh, spreadable ham-and-onion

sausage for 15 day at 8 °C. While a certain level of residual nitrite in cured meat products is crucial for the effectiveness, lower temperatures (0-5 °C), lower product pH (less than 6.0), and reduced oxygen atmosphere can also improve the listeriostatic function of sodium nitrite in cured meat and poultry products (Buchanan and others 1988; Grau and Vanderlinde 1992; Buchanan and Golden 1995). Most recent reports (Pittman and others 2007) have shown that *C. jejuni* can express specific proteins for the protection against the stress introduced by nitrite. Thus, the importance of nitrite for pathogen control is likely to be dependent upon the pathogen involved.

Bacteriocins and antagonistic microorganisms

Bacteriocins: Bacteriocins are biologically active proteinaceous compounds produced by lactic acid bacteria. These compounds demonatrate antimicrobial activity towards foodborne pathogens and spoilage bacteria (De Matinis and others 2002). Nisin, produced by *Lactococcus lactis*, is the most common bacteriocin, and one that has been approved as GRAS and applied to many food products as a preservative (Modi and others 2000). Nisin was reported to have antagonistic effects on gram-positive foodborne pathogens, such as *L. monocytogenes*, *Staphylococcus aureus and Bacillus cereus*. However, meat is not an ideal environment for nisin to fully express antimicrobial functionality. Meat pH, the fat content of meat products and proteolytic activity in raw meat are three factors that limit the efficiency of nisin for inhibition of pathogenic bacteria in meat products; therefore, many studies have attempted to apply nisin to meat products with other hurdles, such as other antimicrobials, modified atmosphere packaging or bacteriophages, or with physical treatments like heating, high pressure or irradiation (Modi and others 2000; Dykes and Moorhead 2002; Matinis and others 2002; Aasen and

others 2003). Samelis and others (2005) observed that when nisin alone was used as a dipping solution for sliced pork bologna, it reduced *L. monocytogenes* by 1.0-1.5 log cfu /cm^2 immediately after dipping and during storage at 4 °C for 10 days, however, the survivors grew back rapidly afterward. When nisin was combined with acetic acid, sodium diacetate or potassium benzoate, the combination treatments inhibited the growth of the pathogen for 90 days of storage. Geonaras and others (2006) also observed that when combining potassium lactate and sodium diacetate as ingredients in the formulation with nisin as a post-process dipping solution, *L. monocytogenes* on frankfurters was reduced by 2.4-3.8 log cfu /cm^2 immediately after dipping, and the cell number was continuously reduced by about 0.5 log during 48 days of storage at 10 °C. Theivendran and others (2006) used nisin combined with grape seed extract or green tea extract as components in a soy protein film forming solution. This combination reduced *L. monocytogenes* by more than 2 log on frankfurters during 28 days of storage at 4 °C or 10 °C.

Studies have shown that combining nisin with other hurdles not only inhibited gram-positive bacteria, but also gram-negative bacteria. Gill and others (1999) combined nisin with lysozyme and EDTA to control the growth of *E. coli O157:H7*, *L. monocytogenes*, *Salmonella* Typhimurium and other gram-negative and gram-positive bacteria on ham and bologna stored at 8 °C for 4 weeks. The combination inhibited the growth of *L. monocytogenes* for 2 weeks, *Salmonella* for 3 weeks and *E. coli O157:H7* for 4 weeks.

In addition to nisin, other bacteriocins, such as pediocins, have demonstrated the pathogen-inhibitory effect. Although pediocin has not been approved to be used in meat products, Chen and others (2004) observed that a commercial food ingredient

ALTA^{TM}2341 sold as a fermentation product, possessed the same antilisterial-function as pediocin. These authors sprayed a solution of ALTA^{TM}2341 on the surface of frankfurters prior to vacuum packaging. The growth of *L. monocytogenes* on frankfurters was inhibited for 7 weeks and the growth rate was significantly reduced for 12 weeks at 4 °C. Uhart and others (2004) combined pediocin, sodium lactate and sodium acetate in a dipping solution for the treatment of beef frankfurters. The numbers of *L. monocytogenes* were reduced by 1.5-2.5 log units during 3 weeks of storage at 4 °C.

Antagonistic lactic acid bacteria: In recent years, extensive studies have been conducted using antagonistic lactic acid bacteria against foodborne pathogens in meat products. Kostrzynska and Bachand (2006) concluded in a comprehensive review that at least four benefits of using these lactic acid bacteria in produce and meat products could be realized. 1. most of the bacteriocin-producing lactic acid bacteria are isolated from fermented or cooked meat products which can satisfy the consumer desire for natural products, 2. many antagonistic bacteria have a wide spectrum of inhibitory properties and can inhibit not only gram-positive, but also gram-negative pathogenic bacteria in meat products, 3. antagonistic bacteria can be used not only in RTE meats, but also in raw meat products, 4. some of the non-bacteriocin producing lactic acid bacteria isolated from meat products can be used as competitive bacteria to inhibit the growth of pathogenic organisms and avoid adapted resistance of the pathogens to bacteriocins. This group of lactic acid bacteria has been used in dry sausage starter cultures to provide bioprotective function against pathogens like *E. coli O157:H7* and *L. monocytogenes*, which can survive the entire multi-hurdle process procedures of salting, curing, fermentation and drying (Tyopponen and others 2003, Benkerroum and others 2005; Alves and others 2006). The protective cultures were also used for control of *E. coli O157:H7* and

Salmonella in ground beef (Smith and others 2005b), and for control of *L. monocytogenes* and *E. coli O157:H7* on pre-cooked sliced ham in vacuum or modified atmosphere packaging (Bredholt and others 1999). While many lactic acid bacteria have been screened for antimicrobial activity (Amezquita and Brashears 2002; Ammor and others 2006), screening for the quality and sensory impact of these organisms on meat products has also been an objective of many studies (Vermeiren and others 2004). However, like many other technologies, there are also concerns for the use of antagonistic bacteria in meat products. Hugas and others (2003) pointed out in a review, that many bacteria which have antibacterial functions also produce biogenic amines. Furthermore, meat-borne enterococci can act as pathogens. Therefore, screening for non-bioamine producers and non-pathogenic enterococci is critical. Lucke (2000) indicated that if genetically engineered cultures are used for this purpose, this will involve additional regulatory events, although many studies have been done showing that engineering of lactic acid bacterial strains to express antimicrobial functions without producing pathogenic activities against humans is very feasible.

Bacteriophage: Studies have reported successful use of bacteriophages on farms for control of colonization and shedding of *E. coli O157:H7* in cattle, and for *Salmonella* and *Campylobacter* in chickens. Greer (2005) reviewed studies and rationales of pre- and post-harvest application of phages in food and meat products. Research has shown that bacteriophages effectively inhibit foodborne pathogens on fruit, vegetables, dairy products and meat (Hudson and others 2006). The Food and Drug Administration has approved a preparation of *Listeria*-specific bacteriophage made from six purified phages as an antilisterial-agent for RTE meat and poultry products in the U.S. (FDA 2006).

Antimicrobials from plants

Essential oils: Essential oils are liquid oils containing phenolic compounds which are extracted from plant-based materials, such as roots, flowers, seeds, leaves, barks, fruits and others (Burt 2004). Essential oils have received a great deal of research attention in recent years because they are natural products and have both antioxidant and antimicrobial functions in various food systems. Essential oils are GRAS according to the federal law (21 CFR 182.20), and are considered as natural flavors. In Burt's review (2004) of antibacterial properties of essential oils, possible modes of action of phenolic compounds in essential oils were described. Phenolic compounds (with hydroxyl groups) in essential oils can degrade bacterial cell walls, damage the cytoplasmic membrane through destruction of membrane protein and cause the leakage of cell contents. Essential oil components also cause coagulation of cytoplasm and depletion of the proton motive force of bacteria cells. Gram-positive bacteria are more sensitive to essential oils then gram-negative bacteria. Among essential oils, rosemary oil (encapsulated), thyme oil or oregano oil (carvacrol and thymol are active compounds), clove oil (eugenol is active compound) and allyl isothiocyanate (antimicrobial contained in many spices and vegetables) were reported to be effective for control of the growth of *Salmonella*, *L. monocytogenes* and *E. coli O157:H7* in fresh or RTE meat products during storage (Burt 2004; Nadarajah and others 2005; Falcone and others 2007; Seaberg and others 2003). Low fat content, reduced pH (less than 6.0), mild heat (45 °C) and reduced oxygen environment (100% CO_2 MAP or vacuum packaging) facilitated the antimicrobial functions of the active compounds (Skandamis and Nychas 2001; Skandamis and others 2002; Burt 2004). Synergistic effects have been reported by many studies when the

combinations of several essential oil active compounds were used; or the application of essential oils was combined with other chemical and physical treatments (Blaszyk and Holly 1998; Tsigarida and others 2000; Chiasson and others 2005; Pranoto and others 2005; Oussalah and others 2006; Ghalfi 2007). Because a high concentration of essential oils is needed for effective control of food pathogens, meat flavor and appearance may be changed by these plant-derived compounds (Burt 2004).

High hydrostatic pressure and other hurdles

Control of foodborne pathogens with high hydrostatic pressure has been very effective for fruit juices, sauces and ready-to-eat meat products. In Patterson's (2005) review, many studies on the use of high pressure (above 100 MegaPascal) to inactivate *E. coli O157:H7*, *Salmonella* and *L. monocytogenes* in fruit juice, milk and meat products were included. The author also described possible modes of action of high pressure. High pressure can damage bacterial cells through physical destruction of cell membranes and cause leakage of cell components, such as ATP, proteins and RNA. High pressure can also cause intracellular damage, disruption of enzyme systems for DNA replication and transcription, and condensation of nuclear materials. In the U.S., high pressure equipment is readily available and is utilized by the meat industry (Hugas and others 2002) for production of commercial meat products. The resistance of foodborne pathogens to high pressure varies significantly between different bacteria and between different strains as well. Patterson and others (1995) estimated the pressure sensitivity of vegetative pathogens in phosphate buffer, ultra high-temperature treated (UHT) milk and poultry meat. The authors reported that some stains of *L. monocytogenes* and *E. coli O157:H7* were more resistant to high pressure than *Salmonella* and other pathogens; *E. coli*

O157:H7 was most sensitive in buffer, while *L. monocytogenes* was most sensitive in poultry meat. These authors also reported that the level of pressure, the temperature during the treatment and the pressure holding time significantly affected that effectiveness of high pressure treatments. A review by Hugas and others (2002) indicated that the efficacy of high pressure was product-dependent and affected by different models of high pressure equipment. Combining high pressure with other hurdles, such as mild heat (48 °C-65 °C), holding time (5 min or longer), bacteriocin (nisin), and reduced product pH, enhanced the bactericidal efficacy of high pressure. However, Igura and others (2003) observed that high pressure (200 MPa) at low temperature (0 °C) for 60 min inactivated *Salmonella* in 0.9% NaCl solution more effectively than at higher temperatures (5 °C or 10 °C); though at the same pressure and temperature condition, *Campylobacter* was more sensitive to high pressure in 0.9% NaCl than on chicken thighs. Further, this treatment changed the color of chicken thighs. Martinez-Rodriguez and Mackey (2005) observed that, although high pressure resistance of *Campylobacter* was different between strains, high pressure was still an effective technology for reducing this pathogen in food. Studies have shown that nisin increased the sensitivity of foodborne pathogens to high pressure (Chung and others 2005). High hydrostatic pressure is more feasible for RTE meat than for raw meat, because this technology can affect the texture and appearance of raw meat products. Although high pressure does not break covalent bonds between molecules, it can change meat quality by increasing water holding capacity, tenderizing meat, decreasing redness of raw meat, increasing firmness and springiness of processed meats (dimerization of proteins), and inducing lipid oxidation (MacFarlane and McKenzie 1976; Cheah and Ledward 1995, 1997; Jimenez-Colmenero

and others 1998; Huang and others 1999; Marcos and others 2005; Ichinoseki and others 2006).

Modified atmosphere packaging and other hurdles

Modified atmosphere packaging (MAP) has been used in the U.S. meat industry for almost thirty years (Rao and Sachindra 2002). Both industry practice and research have demonstrated that modified atmosphere packaging can extend the shelf life of meat products by inhibiting the growth of spoilage microorganisms, and is also effective for control of foodborne pathogenic bacteria (Farber 199; de Fernando and others 1995). As estimated in many studies, about 90% of the boxed beef products in the U.S. are packaged with vacuum or MAP packaging. Case-ready meat and poultry products dominate the market, and meat and poultry packaged in MAP is increasing each year (Eilert 2005). In MAP the most common gas components are carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2). The concentration of each gas in the gas mixture is different as determined by effectiveness for specific applications. High O_2 MAP, for example, contains at least 30-70 % O_2 and 20-30 % CO_2; low O_2 MAP (or high CO_2 MAP) contains less than 20% O_2 and 60-100% CO_2; N_2 is normally used as a filler gas for maintaining the head space of MAP if it is necessary (Jay and others 2005). The main function of O_2 in MAP is to maintain red color of fresh meat (oxygenation of myoglobin) during short term refrigerated display (Gill and Jones 1995; Ho and others 2003). The active gas component for control of bacteria in MAP is CO_2.

Mode of action: Although the mechanism of the bacteriostatic function of CO_2 is still not entirely clear, there are two theories to explain the mode of action. First, CO_2 may disrupt the metabolic functions of bacteria by affecting enzymatic decarboxylation.

Second, when CO_2 is absorbed into the water phase of food products, it forms carbonic acid in water. The ionic form HCO_3^- (also called dissociation form) can change the permeability of bacterial membranes (Jay and others 2005). Gram-negative bacteria are more sensitive to CO_2 than gram-positive. Several factors affect the antimicrobial function of CO_2 in MAP: the CO_2 partial pressure, the initial concentration of CO_2, the initial ratio of gas volume to meat weight, product pH, product fat content, and the storage temperature. It appears that with more CO_2 dissolved in the water phase of the product, there is greater antimicrobial function. Carbon dioxide is more soluble in the water phase at low temperature. Although the solubility of CO_2 is greater at higher pH, a lower pH (< 6.0) facilitates the formation of dissociated carbonic acid, which may be more effective (Gill 1988; Gill and Penney 1988, Devlieghere and others 1998; Jakobsen and Bertelsen 2004).

Control of foodborne pathogens in raw meat and poultry products: Many studies have been done on the growth behavior of foodborne pathogens on meat products packaged in MAP with different gases and at different temperatures. Most of the studies done in the 1980's have shown that on meat and poultry products, the growth of *Salmonella* and *E. coli O157:H7* at minimum or above minimum growth temperatures was effectively retarded by MAP, in a variety of compositions (high or low O_2 MAP) (Luiten and others 1981; Eklund and Jarmund 1983; Backer and others 1986). Gill and Delacy (1991) studied the growth of *Salmonella* Typhimurium and *E. coli* on high-pH beef (pH > 6.0) packaged in MAP (100% CO_2) at a wide range of temperatures. These authors observed that in vacuum packaging, these organisms grew at temperatures from 8 °C to 30 °C; however, in MAP, *Salmonella* did not grow at 10 °C, and *E. coli* did not grow at 9 °C. Between 11 °C to 12 °C, the lag phases of each organism were increased by

3 days, and the growth rates were decreased. At 15 °C, however, the inhibitory effect of CO_2 started to decrease (1 day lag phase), and at 20 °C, no lag phase was observed, and the organisms grew rapidly at the same rate as spoilage bacteria. Nissen and others (2000) studied the growth of several foodborne pathogens at 4 °C and 10 °C on ground beef packaged in MAP with two different gas atmospheres (high O_2 or high CO_2), and also in stuffed chub packs. These authors reported that at 10 °C, the growth of *E. coli O157:H7* was inhibited completely by both high O_2 MAP (70% O_2 / 30% CO_2) and high CO_2 MAP (60% CO_2 / 40% N_2 /0.4% CO); however, after 5 and 7 days of storage at the same temperature, the growth of *Salmonella* was faster in high CO_2 MAP than in high O_2 packaging. The growth of *L. monocytogenes* was inhibited in all three atmospheres at 4 °C; however, *L. monocytogenes* grew in all three atmospheres at 10 °C with about 1 log more in high O_2 MAP or chub packs than in high CO_2 MAP. Michaelsen and others (2006) reported that in MAP (99.6% CO_2 /0.4% CO) inhibited the growth of *Salmonella* on fresh pork chops at 10 °C for 35 days; however, this pathogen grew in vacuum packages after 7 days of storage at the same temperature. These authors also reported that the effect of high CO_2 concentration in MAP was similar to the effect of antimicrobial (2.4 % potassium lactate plus 0.25% sodium diacetate) for control of *Salmonella* in this study. Dykes and others (2001) reported that *E. coli O157:H7* and *Salmonella* on primal beef cuts survived, but did not grow, in both vacuum and 100% CO_2 MAP stored at -1.5 °C for 6 weeks, then at 4 °C for 2 weeks, and suggested that although some studies observed inhibitory effects of CO_2 on these pathogens, these organisms remained a public health risk in CO_2 MAP. Boysen and others (2007) studied the effect of three gas mixtures for control of *C. jejuni* on chicken meat at 4-5 °C, and observed that this pathogen survived better in MAP without oxygen (70% CO_2/ 30% N_2 or 100% N_2) then

with oxygen (70% O_2 /30% CO_2). MAP with oxygen reduced the pathogen at least 4.6 log during 21 days of storage, but only by 0.3-0.8 log in MAP without oxygen. This study confirmed the results of previous studies conducted by Stern and others (1986) and Grigoriadis and others (1997). These authors also observed that packaging in air was more lethal to *Campylobacter* than in CO_2 or vacuum. Carbon dioxide in MAP without oxygen might protect *Campylobacter* from other environmental stressors, such as low temperature (Beuchat 1985); therefore, the poultry industry should consider this information if high CO_2 MAP is used to extend shelf life of poultry products.

Control of pathogens in RTE meat products: Marshall and others (1991) reported that the growth of *L. monocytogenes* on chicken nuggets was delayed for 9 day at 3 °C in both of high CO_2 MAP, with O_2 (76% CO_2 /10.7% O_2 /13.3% N_2), or without O_2 (80% CO_2 /20% N_2). However, when temperature increased to 7 °C, MAP without oxygen had greater inhibitory effect than with oxygen (6 days of lag phase versus 2 days). Kramer and Baumgart (1992) studied the effect of different volumes of CO_2 in MAP for control of *L. monocytogenes* on frankfurter-type sausage at 4 °C or 7 °C. These authors observed that only when the volume of CO_2 reached 80% in MAP, was the growth of the pathogen inhibited at both temperatures. Michaelsen and others (2006) also reported that high CO_2 MAP (100%) inhibited the growth of *L. monocytogenes* on sliced ham for 56 days at 4 °C compared to 28 days in vacuum, and for 35 days at 10 °C compared to 5 days in vacuum.

MAP combined with other methods: Similar to the technologies mentioned above, studies have also attempted to combine MAP with other measures to improve the control of foodborne pathogens in meat and poultry products. These measures include organic acids, nisin, essential oils, irradiation and others. Dipping chicken thighs in 5% potassium

sorbate solution for 1 min was reported by Gray and others (1984) to completely inhibit the growth of *Salmonella* Enteritidis during the storage at 10 °C for 10 days when combining with 100% CO_2 MAP. Reduction in the concentration of CO_2 in MAP or the concentration of potassium sorbate decreased the effectiveness of the treatment. Nilsson and others (1997) applied nisin (500-100 International Units per gram of the product) on cold salmon followed by packaging the product in 100% CO_2-MAP. This method reduced *L. monocytogenes* by 1 to 2 log, and also extended the lag phase of this pathogen from 8 to 20 days when the product was stored at 5 °C. These authors reported that the antilisterial effect of nisin was enhanced by CO_2 in MAP and addition of salt (NaCl) in the products. Tsigarida and others (2001) observed that addition of 0.8% of oregano essential oil to beef packaged in MAP (40% CO2 /30% O2 /30% N2) initially reduced *L. monocytogenes* by 1 log, and the numbers of the pathogen continued to decrease during storage at 5 °C. After 14 days of storage, the pathogen was undetectable, and the total reduction of this organism was 2-3 logs. The authors also reported that CO_2 in MAP did not have an effect in this study, because the pathogen reduction was the same as in vacuum packages. Packaging in impermeable film plus oregano essential oil was concluded to be the main cause of pathogen reduction. Liserre and others (2002) observed a synergistic effect of combining *Lactobacillus sake* (bacteriocin producing organism) with high CO_2 MAP (50-100% CO_2). This treatment reduced *L. monocytogenes* by 3.5 log on Brazilian sausage during 14 days of storage at 6 °C. Djenane and others (2005) also observed a synergistic effect of combining *Lactobacillus sake* with MAP (20-40% CO_2) to control *L. monocytogenes* on beef steaks. This treatment reduced the pathogen 1.5 to 2.5 log during 7 days of storage at 8 °C. During a temperature abuse test at 25 °C, the pathogen was inactivated (5 log reduction from the

initial number) after 5 days of incubation. Al-Haddad and other (2005) reported that treatment with gaseous ozone (>2000 ppm for 30 min) followed by high CO_2 MAP (70% CO_2) initially reduced *Salmonella* on chicken breast by about 1.5 log, and the survivors remained viable for 9 days during storage at 7 °C without further growth. Nadarajah and others (2005a) treated the surface of ground beef patties with allyl isothiocyanate (a natural compound from mustard and other vegetables) followed by packaging in 100% N_2-MAP. This treatment resulted in a greater than 3 log reduction of *E. coli O157:H7* during 21 days of storage at 4 °C, when the contamination level was 6 log cfu /g. A 3 log reduction was observed during 18 days of storage at 4 °C when the contamination level was 3 log cfu /g, or 10 days of storage at 10 °C at the same initial contamination level. The same group of authors (Nadarajah and others 2005b) reported that direct addition of 10 % mustard flour to ground beef followed by packaging the product in 100% N_2-MAP reduced *E. coli O157:H7* by 3 log after 12 days of storage at 4 °C. These authors also reported that the sensory properties of the ground beef with 10% mustard flour was acceptable after cooking. Ellis and others (2006) placed fast or slow release chlorine dioxide sachets in MAP containing 100% N_2 or 75% N_2 /25% CO_2 for the packaging of fresh chicken breasts. This method reduced *Salmonella* by 1.0 to 1.5 log during refrigerated storage for 15 days. However, the color of chicken breasts close to the sachets became more yellow. No off-odor was detected in the product.

Because many of the RTE meat products contain organic acid salts (particularly sodium or potassium lactate), a predictive model was developed by Devlieghere and others (2001) to determine the interactive effect of the concentration of sodium lactate, water activity of the product, concentration of CO_2 absorbed in the product and storage temperature for control of *Listeria monocytogenes* in pre-cooked meat products. The

model shows that the interaction between temperature and CO_2, and between CO_2 and lactate are the main interactive effects of these combined measures for control of the pathogen in RTE meat products.

Shelf life of meat and poultry packaged in MAP: Modified atmosphere packaging was originally developed for extension of meat product shelf life by control of spoilage microorganisms. Since meat products with high water activity are normally stored at refrigeration temperatures, the spoilage bacteria that can grow under these conditions are psychrotrophs. *Pseudomonas* spp are predominant spoilage bacteria in aerobic packaging of fresh meat, and lactic acid bacteria predominate in vacuum or anoxic packaging, such as 100% CO_2-MAP (de Fernando and others 1995; Devlieghere and others 1998) of fresh meat or on meat products with salt added. Therefore, the original purpose of CO_2 in MAP was for control of psychrotrophic microorganisms (Tewari and others 1998; Venturini and others 2006). Shelf life is not only related to the growth of spoilage bacteria, but also to quality changes of the product during the storage, and includes meat color stability, oxidative stability, package purge, product pH, and sensory attributes. Many intrinsic and extrinsic factors affect the shelf life of meat and poultry packaged in MAP, including the type and origin of the product, the history of the product, the type and amount of spoilage bacteria at the initial stage, different gas compositions in MAP with different hurdles, different storage temperature, packaging materials, pH and water activity, the amount of CO_2 dissolved in products and many others. For example, Bailey and others (1979) reported that lower temperature, higher concentration of CO_2 and high barrier packaging film contributed to lower microbial count on chicken packaged in MAP. Blickstad and Molin (1983) observed that combining 100% CO_2-MAP with low temperature (0 °C) extended the shelf life of fresh

pork loins for 3 months, and 5 months for cured pork loins. Devlieghere and others (2000) reported that sodium lactate and the amount of CO_2 synergistically inhibited the growth of spoilage bacteria in cooked meat products, and this synergistic effect was facilitated by low temperature.

In a study by Hotchkiss and others (1985), MAP with high CO_2 inhibited the growth of gram-negative aerobes during 14 to 35 days of storage at 2 °C. In this study, the microflora on chicken quarters was shifted during storage to gram-positive facultative or anaerobes as dominant bacteria. Fu and others (1992) observed that MAP (10 or 20% O_2 and 20 or 40% CO_2) did not suppress the growth of facultative anaerobes on beef rib eye steaks when the product was stored at 2°-4 °C for 7 weeks. Oxygen concentration decreased while CO_2 increased in MAP over time. The lag phase of *Enterobacteriaceae* on the product was extended for 1 week in MAP packaging. Jackson and others (1992) reported that, in MAP containing more the 80% O_2 and 20% CO_2, *Pseudomonas* spoiled beef strip loins after one or two weeks of storage at 3 °C as evidenced by strong off-odors. Lactic acid bacteria were the dominant microflora in vacuum packaging, and MAP with 100% CO_2 or 40%CO_2 plus 60% N_2. There was much less off-odor produced in these packages, however, acidic and sour odors were detected in the high CO_2 MAP packages. Jeremiah and others (1992) observed that 100% CO_2 in MAP extended the shelf life of chilled pork for 24 weeks, which was longer than the product packaged in vacuum (18 weeks). Berruga and others (2005a) reported that with four different gas mixtures in a MAP (high O_2 + low CO_2, low O_2 + low CO_2 + N_2, CO_2 + N_2 and high CO_2 + low O_2) inhibited the growth of spoilage bacteria (lactic acid bacteria, *Pseudomonas* and *Enterobacteriaceae*) on rabbit carcasses. Berruga and others (2005b) also reported that, compared to MAP, bacterial counts were much higher on lamb meat packaged in vacuum.

For pre-cooked meat and poultry products, O_2 is usually not used in MAP. Patsias and others (2006) observed that high CO_2 MAP was more effective than aerobic packaging for extension of shelf life of pre-cooked chicken meat. In this study, the growth of spoilage bacteria on chicken breast fillets (packaged in MAP with 60 to 90 % CO_2) was inhibited for one week in comparison to aerobic packaging.

Combining MAP with other methods was also effective for control of psychrotrophic spoilage bacteria in meat and poultry. Jimenez and others (1999) used 1% acetic acid as a dipping solution for decontamination of chicken breast portions (dipped for 1 min) before packaging the product in high CO_2 MAP (70% CO_2 /30%N_2), and observed that combining these two methods inhibited the growth of psychrotrophs in chicken meat for 14 to 21 days during storage at 4 °C in comparison to 6-8 days for the product untreated with acetic acid. Gill and Badoni (2003) also reported that pasteurizing manufacturing beef in hot water for 1 min before packaging in high O_2 MAP (70% O_2 / 30% CO_2) maintained a lower number of spoilage bacteria in the product during 12 days of storage at 2 °C, and during 3 days of display at 4 °C in comparison to the unpasteurized product. Skandamis and Nychas (2001) reported that addition of oregano essential oil (0.5-1.0%) to minced beef before packaging in MAP, reduced the total plate count by 2-3 log and extended the product shelf life by 12 days during storage at 5 °C in comparison to 5 days in aerobic packaging.

The color stability of meat and poultry packaged in MAP: One of the challenges for MAP is that CO_2 in MAP may discolor fresh meat products during storage. Hotchkiss and others (1985) observed that the color score for chicken quarters packaged in 80% CO_2-MAP was lower than chicken in 60 or 70% CO_2-MAP after 35 days of storage. Rousset and Renerre (1991) reported a purple-red color for beef with normal pH when

the product was packaged in 100% CO_2 MAP. This has been typical for beef packaged in vacuum. Therefore, O_2 has been used in MAP to retain the red color (bloom) that is most desirable by producing oxymyoglobin. Fu and others (1992) observed that least 20% O_2 was necessary for maintaining the red color of beef, however, the red color faded and yellowness increased during storage. A model developed by Jakobsen and Bertelsen (2000) predicted that 55 to 80% O_2 would be necessary for MAP-packaged meat to retain redness for 10 days at refrigeration temperature (lower than 4 °C). Berruga and others (2005b) reported that the redness (a* value) of lamb meat packaged in CO_2 MAP (40% /60% N2, 80% CO2 /20% N2 or 20% O_2 /80% CO_2) decreased significantly during 28 days of storage at 2 °C, in comparison with lamb meat packaged in vacuum. Penney and Bell (1993) and Venturini and others (2006) suggested that one of reasons for discoloration of fresh meat packaged in high CO_2 MAP during storage was residual oxygen in the packages. With a level of 0.05-0.1% residual oxygen under the partial pressure created in MAP or vacuum packaging, deoxymoglobin is rapidly oxidized to metmyoglobin, which contributes to the browning of meat products. However, this problem can be mitigated by placing oxygen scavengers in MAP.

Package purge and pH of meat and poultry packaged in MAP: Because CO_2 in MAP can form carbonic acid by reaction with water in meat and poultry products, lower pH is generally expected in meat packaged in high CO_2 MAP. Holley and others (1994) and Jakobsen and Bertelsen (2002) suggested that although high concentration of CO_2 in MAP may decrease the surface pH of meat products and affect some chemical qualities, no detrimental quality effect is likely. Sorheim and others (2004) observed that high CO_2 MAP decreased pH of ground beef by 0.12 units in comparison to the product packaged in N_2 MAP or vacuum. The authors suggested that the higher cooking loss of ground beef

from high CO_2 MAP might be related to lower pH and porosity in ground beef caused by CO_2. Vergara and others (2005) reported that slightly though not significantly lower pH was observed in rabbit meat packaged in high CO_2 MAP, and that the rabbit meat had significantly greater cooking loss. Michaelsen and others (2006) also reported that pork chops packaged in high CO_2 MAP had significant drip loss (purge) during storage at 4 °C for 35 days, although pH of the product was not reported. Devlieghere and others (2000) suggested that the pH-decreasing effect of CO_2 contributed to synergistic effects between sodium lactate and CO_2 for control of bacteria in pre-cooked meat and poultry products packaged in MAP.

Lipid oxidation in meat and poultry packaged in MAP: Compared to vacuum packaging, MAP with CO_2 and O_2 may promote lipid oxidation, with the higher concentration of O_2 used in MAP, favoring greater lipid oxidation (Fu and others 1992). Berruga and others (2005a) and Berruga (2005b) also reported that O_2 in MAP (with both high and low concentration) promoted lipid oxidation in rabbit and lamb meat during cold storage at 1 ° or 2 °C. Huang and others (2005) reported that dipping pork chops in ascorbic acid (500 ppm) or in citric acid (250 ppm) reduced lipid oxidation in the product when pork chops were first packaged in high CO_2 MAP and then moved to high O_2 MAP after one week of storage at 1 °C. Patsias and others (2006) reported that oxidation stability of pre-cooked chicken fillets packaged in high CO_2 MAP (60-90% CO_2) were greatest in comparison to aerobic packaging. The thiobarbituric values for the chicken meat were far below the rancidity level for at least 8 days during refrigerated storage. Martinez and others (2005) reported that high concentration of CO_2 in MAP also promoted oxidation of lipids and myoglobin. These authors suggested that the pH-decrease effect of CO_2 might contribute the oxidation in pork sausage packaged in MAP.

Change of sensory attributes of meat and poultry packaged in MAP: Hotchkiss and others (1985) reported that, in comparison to fresh chicken meat, chicken quarters packaged in high CO_2 MAP (60-80% CO_2) and stored for 35 days at 2 °C received higher sensory scores for odor, appearance and overall acceptability, but not for color. After the chicken meat was baked, the meat from MAP stored for 28 days received nearly equal scores to fresh chicken for appearance, tenderness, flavor and overall acceptability; however, after 35 days of storage, chicken meat from MAP was less tender, less juicy and had less chicken flavor than fresh chicken meat. Jeremiah and others (1992) observed that chilled pork packaged in high CO_2 MAP became drier over time in storage. Bruce and others (1996) reported that CO_2 that was absorbed in beef packaged in MAP was released during heating and formed pores in the meat. Sorheim and others (2004) also reported that there were fissures and pores formed in cooked ground beef due to the evolution of CO_2 from meat during cooking. Jakobsen and Bertelsen (2002) suggested that the porosity effect of CO_2 appeared more frequently when high concentration CO_2 was used in MAP.

During the cooking process, meat packaged in high oxygen MAP is more likely to be undercooked because the predominant oxymyoglobin pigment is easier to denature. The meat may become prematurely brown before the internal temperature reaches 71.1°C. On the other hand, meat packaged in ultra-low oxygen MAP had potential to be overcooked because the predominant deoxymoglobin pigment is not as easily denatured and may be still be pink after the required internal temperature is reached. The former can be a food safety concern, and the latter is likely to affect meat texture and tenderness (Seyfert and others 2004a. 2004b; John and others 2005).

Carbon monoxide MAP: As it mentioned above, O_2 has been used in MAP for generating bright cherry-red color by reacting with myoglobin in fresh meat products facilitate the bloom color effect. However, O_2 may also contribute detrimental quality effects to meat products in MAP by promoting both lipid and myoglobin oxidation so that shelf life of meat packaged in MAP is shortened. Carbon monoxide became an option for MAP technology when it was approved for applications in the amount 0.4%. This gas had been used for retail meat packaging in Norway since 1985 (Sorheim and others 1997, 1999). Carbon monoxide with concentration below 1% has little antimicrobial effect in MAP (Gee and Brown 1981), but forms bright cherry-red carboxymyoglobin by reacting with myglobin in meat. Carboxymyoglobin results in very similar red color to oxymoglobin, and is much more stable than the latter (Sorheim and others 1997). Another advantage is that CO in MAP can delay the formation of metmyoglobin, and consequently delays lipid oxidation in meat products packaged in CO MAP (Luno and others 2000). Therefore, CO is a very desirable gas component for use in MAP for fresh meat. In 2004, U.S. Food and Drug Administration approved CO as a GRAS substance for use as a component of MAP systems for case-ready fresh beef and pork (FDA 2004). Many studies have been done on use of CO in MAP for meat products. Viana and others (2005) reported that when comparing MAP systems (high CO_2, high O_2, high CO and high CO_2 + low CO) for packaging fresh pork loins, the color values (L* and a*) of the product packaged in MAP with 99% CO_2 + 1% CO remained most similar to the color values of fresh meat during 20 days of storage, and the growth of spoilage bacteria was delayed for 5-10 days at 5 °C. Krause and others (2003) also reported that CO in MAP (20% CO_2 /80% N2 /0.5% CO) maintained greater a* values for pork loins during 36 days of display at 0 °-2 °C in comparison to pork loins packaged aerobically. John and

others (2005) reported that when 0.4% CO instead of high O_2 was used in MAP for packaging of top sirloin steaks, not only was the red color stable during 21 days of storage at 2 °C, but the TBA values were also much lower (TBA=0.7) in CO MAP than in high O_2 MAP (TBA=2.3). Furthermore, the temperature for denaturing carboxymoyglobin was higher than for oxymyoglobin, therefore pre-mature browning was prevented by replacing high O_2 MAP with CO MAP or vacuum.

Food irradiation

Irradiation is another well-established, non-thermal technology that has proved to be effective for eliminating of foodborne pathogens from various food products without detrimentally altering food quality. Irradiation was approved by USDA for control of foodborne pathogens in fresh meat and poultry product (9 CFR Parts 317, 318, 320 and 381; 21 CFR 178.26), and has been used by meat industry for eliminating *E. coli O157:H7* in ground beef (Ross and Engeljohn 2000). Extensive studies have been done on application of this technology for various RTE meat and poultry products, though this technology has not been approved by FDA for the application to cooked meat and poultry products. Many scientific reviews have included details about irradiation including the history, resources, applications, modes of action, resistance and behavior of bacteria in response to irradiation, quality of irradiated meat and poultry and consumer acceptance (Murano 1995; Molins 2000). The content in this review focuses on recent studies of irradiation for control of *L. monocytogenes*, *E. coli O157:H7*, *Salmonella* and *Campylobacter* alone and in combination with other hurdles for both fresh and cooked meat and poultry products.

Mode of Action: The most common application of irradiation is for pasteurization purposes using low or medium-dose gamma ray irradiation (cobalt-60) or accelerated electron beams (0.5-2.5 kGy). The energy released from the isotopic elements or from electrons randomly strikes bacterial genetic material such as DNA and creates lesions on single or double strands of DNA, consequently, causing bacterial cells to lose normal cellular functions and die. Radiation energy also reacts with the water phase in the cell plasma and produces hydroxyl radicals or hydrogen peroxide. These high oxidative materials also react with cell genetic material and cause malfunction or death of the cell. Irradiation also affects cell membranes, enzymes and other functional components in bacterial cells (Dickson 2001).

Radiation sensitivity of foodborne pathogens in meat and poultry: The sensitivity of bacteria toward irradiation is expressed by D_{10}-values, which are defined as the radiation energy needed for reduction of 90% (1 log) of the bacterial cells present. D_{10}-values can be calculated as the negative reciprocal of the slope of a regression plot constructed with irradiation doses as the independent variable, and the number of survival bacteria cells (logarithm) as the dependent variable (Dickson, 2001). Radiation sensitivity of bacteria is affected by many intrinsic and extrinsic factors, such as the structure of bacterial cells, different bacterial strains, environmental pH, temperature, water activity, fat content, different types of meat products, ingredients and antimicrobials in product formulations, product packaging types and others (Thayer and others 1995; Buchanan and others 1999; Sommers and others 2004; Black and Jaczynski 2006). Normally, gram-positive bacteria are more resistant to irradiation than gram-negative bacteria; further, bacteria are more sensitive to irradiation at refrigeration temperatures than at frozen temperatures (Jay and others 2005). According to the final rule issued by the FSIS

(1999b), the maximum dose for pasteurization of refrigerated uncooked meat and poultry product is 4.5 kGy, and for frozen uncooked meat the maximum dose is 7.0 kGy. There are also D_{10}-values for several key foodborne pathogens presented in the final rule for general references: 0.25 kGy (refrigerated products) to 0.45 kGy (for frozen products) for *E. coli O157:H7*, 0.4 to 0.64 for *L. monocytogenes*, 0.48 to 0.70 for *Salmonella* and 0.18 kGy to 0.24 kGy for *C. jejuni*. Niemira and Solomon (2005) observed that biofilm-related cells of *Salmonella enterica* serovars were more sensitive to irradiation than the plantonic cells. Buchanan and others (1999) reported that acid adaptation of *E. coli O157:H7* increased resistance of this pathogen to irradiation. Thayer and Boyd (1993) observed that *E. coli O157:H7* on chicken meat was more sensitive to irradiation than on lean beef. Thayer and others (1998) reported that *L. monocytogenes* on cooked meat survived irradiation better than on raw meat products. Sommers and Thayer (2000) reported that *L. monocytogenes* on frankfurters made from meat originating from one animal was more sensitive to irradiation than on frankfurters made from blended meat sources. These authors also reported that non-meat ingredients, including salt, soy flour or soy protein protected the pathogen from irradiation destruction. Krishnamurthy and others (2004) reported that packaging type and packaging materials affected the radiation sensitivity of *E. coli O157:H7* and *Salmonella*. These authors suggested that *E. coli O157:H7* was more sensitive to irradiation in packaging with low-density polyethylene (LDPE) film, and *Salmonella* was more sensitive to irradiation in packaging with polylactic acid film, if the temperature and the irradiation dose were consistent. Thayer and Boyd (1991) reported that *Salmonella* Typhimurium on mechanically deboned chicken meat was more sensitive to irradiation in aerobic packaging than in vacuum packaging. Jo and others (2004) observed that the resistance of *E. coli O157:H7* to irradiation was increased by

marinating of beef. Clavero and others (1994) reported that *C. jejuni* on low-fat ground beef was more resistant to irradiation than on the high-fat product. Sherry and others (2004) used 40 *Salmonella* serovars to test the stress resistance of the pathogen and observed that each serovar had a different sensitivity toward irradiation. These authors suggested that choosing a representative serovar for modeling the radiation sensitivity of this pathogen is crucial for the accuracy of risk assessment. Therefore, to address the radiation sensitivity of foodborne pathogens in meat and poultry products, more information on these factors will make the data more useful for industry practices and future studies.

To increase the sensitivity of foodborne pathogens to irradiation or to prevent the growth of irradiation survivors, many studies have attempted to combine irradiation with other control measures. Sommers and others (2002) used vauum-steam-vacuum treatments in sequence to decontaminate hams prior to irradiation treatment. These authors reported that this combination reduced *L. innocua* on the product by 4.4-4.8 log with 2.0 kGy of irradiation. The additive effect of these two methods was obvious. Sommers and others (2003) observed that addition of sodium diacetate (0.15%) and potassium lactate (2%) decreased the radiation resistance of *L. monocytogenes* on beef bologna by 18%. The combination of irradiation with sodium diacetate and potassium lactate also extended the lag phase of the pathogen for 8 weeks at 9 °C. Chen and others (2004) reported that the combination of irradiation with pediocin applied by dipping not only increased the radiation sensitivity of *L. monocytogenes* on frankfurters, but also extended the lag phase of the pathogen for 12 weeks at 4 ° and 10 °C. Borsa and others (2004) observed that addition of essential oil containing carvacrol, thymol and trans-

cinnamaldehyde increased the radiation sensitivity *of E. coli O157:H7* and *Salmonella* in ground beef.

***Quality and sensory effects of irradiation on meat and poultry products*:** It has been shown by many studies that irradiation affected at least three quality aspects of meat and poultry products: color, oxidative stability and off-odor. Irradiation decreased redness of fresh beef and increased the brownness, but induced redness in fresh or cooked pork and poultry (Nanke and others 1999). Nam and Ahn (2002) attributed the reddening effect to irradiation generated CO in meat binding to the sixth position of iron in myoglobin, and suggested that this reaction was facilitated by the low redox potential in vacuum packaging. Irradiation-induced lipid oxidation has been observed in meat and poultry, especially in products packaged aerobically (Luchsinger and others 1996, Ahn and others 2000). The oxidation induced by irradiation might be the reason for decreased cured color of irradiated RTE meat products (Houser and others 2005). Irradiation has been shown to produce volatiles in irradiated meat and poultry products, which include sulfur and carbonyl compounds that are responsible for irradiated off-odor (Kim and others 2002). Many combination treatments have been studied in attempt to prevent these irradiation defects in irradiated meat and poultry. Fan and others (2006) reported that dipping turkey bologna in a solution of rosemary extract (0.75%) for 2 min prior to irradiation treatment prevented the redness in the product compared to the undipped product. Zhu and others (2004) observed that addition of sodium lactate also reduced irradiation-induced redness in turkey rolls. Du and Ahn (2002) observed that addition of Vitamin E, gallic acid, rosemary extract or sesamol also effectively reduced the redness of irradiated turkey thigh meat. However, these antioxidants were not effective for reducing irradiated off-odor in these products. Fan and others (2004) reported that

rosemary extract effectively reduced the redness on irradiated turkey bologna and controlled the lipid oxidation; however, no effect of this antioxidant on the volatile compounds was observed. Formanek and others (2003) added rosemary extract or α-tocopheryl acetate to minced beef prior to irradiation and observed that these antioxidants resulted in more redness on irradiated minced beef, probably by decreasing metmyoglobin formation. Lee and Ahn (2005) added plum extract (<2 %) to turkey breast rolls prior to irradiation and reported that this compound controlled lipid oxidation in the product and decreased some volatiles (aldehydes) production; however, plum extract also made product appear dark red.

Combination of irradiation and modified atmosphere packaging: Because of the bacteriostatic properties of MAP and bactericidal effects of irradiation, numerous studies have been initiated investigating the combination of these two control measures for a promising bacteria control strategy for meat and poultry products (Lee and others 1996). Patterson (1988) reported that *Salmonella* and *E. coli* on minced chicken meat packaged in 100% CO_2 were more sensitive to irradiation than in aerobic packaging. These authors also reported that *E. coli O157:H7* on ground beef in aerobic conditions or in MAP with low CO_2 (30%) and high O_2 (60%) was more sensitive to irradiation than in vacuum or high CO_2 MAP packaging. Thayer and Boyd (1999) reported that irradiation was more lethal to *L. monocytogenes* in turkey meat packaged aerobically or in MAP with low oxygen concentration than in 100% CO_2-MAP; however, high CO_2 inhibited the growth of survivors of the pathogen during 28 days of storage at 7 °C. Grant and Patterson (1991) observed that the combination of irradiation (1.75 kGy) with MAP utilizing 25% CO_2 /75 % N_2 produced more stable fresh color and less spoilage in pork chops in comparison to the aerobically packaged product. Chiasson and others (2005) observed that the radiation

sensitivity of *Salmonella* Typhimium in ground beef was similar to that in vacuum and in 100% CO_2 MAP packages. These authors reported that *Salmonella* Typhimurium in ground beef was more sensitive to irradiation when packaged in vacuum or 100% CO_2 MAP than when packaged in air. However, in a previous study by Chiasson et al. (2004), *Salmonella* Typhimurium on ground beef showed greater sensitivity to irradiation in MAP containing oxygen (60% O_2 /30% CO_2 /10% N_2) than in vacuum packaging. Kusmider and others (2002) reported that packaged ground beef in MAP + CO (0.5% CO / 70% CO_2 /29.5% N_2) stabilized the bright cherry-red color of the product during irradiation treatment at 2.0 kGy and 4.5 kGy, and during the display at 0 °C-2 °C for 28 days. The TBA values of the product in this study were far below the rancidity level.

HYPOTHESIS FOR THE STUDY AND DISSERTATION ORGANIZATION

The hypotheses for this study is that the combination of irradiation with high CO_2 packaging will be at least as effective as irradiation with vacuum packaging for control of foodborne pathogens in meat and poultry products, and the addition of CO to MAP will result in significant color retention advantages for irradiated fresh meat products. The study included *E. coli O157:H7* in ground beef; *L. monocytogenes* on frankfurters and pre-cooked pork chops, and *Salmonella* and *Campylobacter* on fresh chicken breasts. This dissertation includes an abstract, a general review of literature, three manuscripts prepared for publication in the format required by the Journal of Food Science, Meat Science and Journal of Food Protection. All three manuscripts represent the work done by the first author to fulfill requirements for the degree of Doctor of Philosophy.

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CHAPTER 2—CONTROL OF *ESCHERICHIA COLI O157:H7* IN GROUND BEEF PATTIES BY IRRADIATION COMBINED WITH MODIFIED ATMOSPHERE PACKAGING

A paper to be submitted to the Journal of Food Science

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ABSTRACT

The efficacy of controlling *Escherichia coli O157:H7* in ground beef patties by irradiation combined with high CO_2 (99.5%) and low CO (0.5%) modified atmosphere packaging (MAP) was investigated. Ground beef patties were inoculated with a five strain cocktail of *E. coli O157:H7* (5 log / gram). Single patties, packaged in

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vacuum or MAP, were irradiated at 0 (control), 0.5, 1.0 or 1.5 kGy. The radiation D_{10}-value for this pathogen was 0.50 ± 0.02 kGy in MAP and 0.47 ± 0.02 kGy in vacuum packaging. There was no significant growth, but survival of *E. coli o157:H7* was observed in all packages during six weeks of refrigerated storage, irrespective of irradiation doses. A temperature abuse test (25 ºC) for 48 hours showed 1-3 log growth in vacuum packages irradiated at 1.5 kGy, and 1-3 log growth in MAP packages irradiated at 1.0 or 1.5 kGy. Red ground beef color that is normally affected by irradiation, remained stable in MAP due to addition of CO. Oxidative rancidity, pH and package purge for the ground beef were similar with both packaging types. Sensory evaluation with a 10-member trained panel showed more irradiated off-odor and off-flavor, and less beef flavor for cooked beef patties from MAP packages than from vacuum. This study demonstrated that combining irradiation with high CO_2 MAP + CO was similar to irradiation with vacuum packaging for control of *E. coli O157:H7* in ground beef patties, though more off-odor in MAP packages was observed. A significant advantage of high CO_2 MAP + CO packaging was improved fresh meat color when the irradiation was used to reduce *E. coli O157:H7* in ground beef.

Key Words: Irradiation, *E. coli O157:H7*, modified atmosphere packaging, CO_2, CO, ground beef patties

INTRODUCTION

In 2005, the Centers for Disease Control and Prevention (CDC) reported that cases caused by *Escherichia coli O157:H7* met the National Healthy People 2010 goal (U.S. Department of Health and Human Services 2000; USDA News Release 2005) for

the first time since 1994 when the United State Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) first issued the zero tolerance rule for this pathogen in raw ground beef (9 CFR Chapter III, Docket No. 97-068N). The goal was 1 infectious case per 100,000 persons. The U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) has also reported that *E. coli O157:H7*-positive ground beef samples decreased by 43.3% in 2004 compared to the previous year. Recalls of ground beef contaminated with *E. coli O157:H7* declined from 21 in 2002 to 12 in 2003, 6 in 2004 (USDA-FSIS News Release 2005; Naugle and others 2006), 6 in 2005 and 8 by November, 2006 (USDA-FSIS Recalls 2006). Despite the past successes, continuous commitment is still necessary to further reduce the food-borne illnesses caused by this pathogen.

Many intervention strategies have been developed and are utilized in the beef industry for controlling *E. coli O157:H7* contamination of beef products. Combined with the implementation of Hazard Analysis and Critical Control Point (HACCP) systems, physical and chemical decontamination methods have been extensively studied and applied. For decontaminating beef carcasses, physical methods include trimming, vacuuming with hot steam or hot water, hot water washing, hot steam pasteurization and chilling immediately after the carcass washing (Gill and Landers 2003). Chemical interventions include spraying carcasses with organic acids before or after chilling; spraying carcasses with acidified sodium chloride, hydrogen peroxide, trisodium phosphate or cetylpyridinium chloride (Smulders and Greere 1998; Kang and others 2001a, Castillo and others 2001; Calicioglu and others 2002; Gill and Landers 2003). However, the effectiveness of these methods has been inconsistent. For instance, after investigating four beef slaughter plants, Gill and Landers (2003) reported that among all

physical and chemical decontamination methods, only pasteurization with steam or hot water was effective for reducing the pathogen numbers on beef carcasses. Carcass washing with 2% lactic acid, vacuum-hot water cleaning or trimming procedures were not always effective. Brackett and others (1994) reported that rinsing beef sirloins with hot or warm organic acids did not reduce inoculated *E. coli O157:H7*. Berry and Cutter (2000) indicated that acid-adapted *E. coli O157:H7* was resistant to 2% acetic acid used for beef carcass spraying. Smulders and Greer (1998) also indicated that *E. coli O157:H7* was acid resistant, can attach to beef carcasses tightly, and that organic acid solutions from 1-3% were not effective for elimination of this pathogen. Because of the inconsistent efficacy of decontamination methods used in the beef harvest industry, additional control measures are important consideration if this pathogen is to be eliminated from beef products.

Most of the control measures studied or applied for control of *E. coli O157:H7* in ground beef are hurdle interventions, meaning that two or more methods are combined to achieve additive or synergistic effects for control of bacteria, and to retain or enhance the product quality at the same time (Kang and others 2001b; Patterson 2001; Edwards and Fung 2006). Food irradiation is a well-established technology for eliminating food-borne pathogens in meat products (Olson 1995). However, irradiation, especially at medium doses (1.5 to 2.0 kGy), may also cause quality changes, such as off-odor, changed meat color or lipid oxidation (Fu and others 1995; Nanke and others 1998; Montgomery and others 2000; Nanke and others 1999; Kim and others 2002). These quality changes have limited the consumer acceptance of commercialized irradiated fresh meat products (Luchsinger and others 1996a). Therefore; many studies have been conducted on combination of other hurdles with irradiation to minimize irradiation dose and irradiation

side effects without compromising the safety of the meat products. One of the approaches studied was the combination of irradiation with modified atmosphere packaging (Lee and others 1996). Modified atmosphere packaging (MAP) with either low (20-30%) or high (60-100%) carbon dioxide content has been used for inhibiting spoilage bacteria in meat and to extend the shelf life (Rao and Sachindra 2002). Many reports have shown that MAP with high CO_2 is more effective than low CO_2 for control of spoilage bacteria, and that meat shelf life was longer in MAP with high CO_2 (Blickstad and Molin 1983; Jackson and others 1992; Buys and others 1994; Holley and others 1994; Tewari and others 1999; Berruga and others 2005a). High CO_2 MAP has been reported to inhibit the growth of *E. coli O157:H7*, *Salmonella*, *Listeria monocytogenes* and *Campylobacter jejuni* in meat products (Farber 1991; Nissen and others 2000; Rao and Sachindra 2002; Michaelsen and others 2006). A few studies have combined irradiation with modified atmosphere packaging to control food-borne pathogens in the meat products. Patterson (1988) reported that *Salmonella* Typhimurium and *E. coli O157:H7* in minced chicken meat were more sensitive to irradiation in MAP with 100% CO_2 than in aerobic packages. However, Chiasson and others (2005) observed that when ground beef was packaged in high O_2 MAP (30% CO_2, 60% O_2 and 10% N_2), *E. coli O157:H7* and *Salmonella* Typhimurium were more sensitive to irradiation than in ultra-low O_2 MAP (100% CO_2) or in vacuum packages. The current practice for irradiation of fresh meat is to include vacuum packaging to reduce lipid oxidation by eliminating oxygen from packages when the meat product is subjected to irradiation (Luchsinger and others 1996b; Ahn and others 2001). Therefore, combining irradiation with low oxygen and high CO_2 MAP might be another hurdle intervention strategy to control pathogens in meat products without affecting meat quality. However, high CO_2 MAP packaging can cause meat discoloration

at concentration above 30%-40% CO_2. Further, if high CO_2 MAP contains residual oxygen, the fresh meat color will deteriorate even faster during storage (Penney and Bell 1993; Buys and others 1994; Jakobsen and Bertelsen 2002; Berruga and others 2005b; Venturini and others 2006).

Because the Food and Drug Administration (FDA) approved carbon monoxide (0.4%) as a MAP gas (FDA, 2002), it is feasible to explore the effectiveness of combining irradiation with high CO_2 / low CO MAP to control *E. coli O157:H7* in ground beef without concern for the meat color deterioration problem caused by irradiation or high concentration of CO_2 alone. Carbon monoxide reacts with meat myoglobin to produce bright cherry-red carboxymyoglobin with greater color stability than oxymyoglobin (Luno and others 1998). Kusmider and others (2002) demonstrated that MAP with CO increased red color of irradiated (2 or 4.5 kGy) ground beef. Sensory evaluation results showed that less off-odor was produced in MAP + CO packaging than for irradiated ground beef in vacuum packaging. Consumer tests (Viana and others 2005) showed that pork loins treated with a low level of CO and high CO_2 (1% CO, 99% CO_2) had the most favorable color value compared with the product packaged in 100% CO_2, 100% O_2 or 100% CO. Despite considerable research on irradiation, few studies have been done to evaluate the effect of irradiation on food-borne pathogens in meat products packaged in MAP with high concentration of CO_2 (99.5%) and low CO (0.5%). Further, few studies have investigated the survival of *E. coli O157:H7* on irradiated meat products packaged in the atmosphere with high CO_2 + low CO at refrigeration temperature or with temperature abuse.

The objective of this study was to test the hypothesis that irradiation combined with high CO_2 MAP + low CO is at least as effective as irradiation with vacuum

packaging for reducing *E. coli O157:H7* in fresh ground beef patties, and for inhibiting the growth of survivors during temperature abuse, as well as superior to vacuum packaging for meat color. Quality evaluations (oxidative rancidity, pH and package purge) and sensory evaluations (color, off-odor, sour-like aroma, and beef aroma for raw beef patties; sour-like aroma, ground beef aroma, juiciness and sourness for cooked patties) were also included in this study to determine the over-all feasibility of the combined hurdles.

MATERIALS AND METHODS

Experimental design

This study was conducted as two experiments. The microbiology assessment was done in experiment 1 while the product quality effects were evaluated in experiment 2. A random block design was used for both experiments. A 2 × 4 factorial design was used for treatments in experiment 1 to determine radiation D_{10}-values of *E. coli O157: H7* in ground beef and to assess the survivor growth status during storage. Vacuum and modified atmosphere packaging (MAP) comprised two levels of packaging while irradiation doses of 0 kGy (control), 0.5 kGy, 1.0 kGy and 1.5 kGy comprised four levels of irradiation dose. A 2 × 3 factorial design was used for sensory evaluation in experiment 2. Vacuum and MAP comprised two levels of packaging with irradiation doses of 0 kGy (control), 1.0 kGy and 1.5 kGy as three levels of irradiation dose. A 2 × 3 × 2 factorial design was used for color, purge, pH and rancidity evaluations. The two packaging treatments (vacuum and MAP), three irradiation doses (0 kGy, 1.0 kGy and 1.5 kGy) and two storage times (first day and 7^{th} day after irradiation) were used for these assessments. There were three samples analyzed for each treatment in experiment 1 and

two samples for each treatment in experiment 2. Both experiments were repeated three times.

Experiment 1: *Experiment 1 was designed to determine and compare the irradiation reduction rates (D_{10}-value) for E. coli O157:H7 in beef patties packaged with either vacuum or high CO_2 MAP packaging, and to evaluate the fate of survivors during storage at 2-4 ºC and following temperature abuse at 25 ºC.*

Preparation of meat samples

Refrigerated fresh beef chuck roasts (vacuum packaged) purchased from a local supplier were used to manufacture commercial-sized beef patties (1.0 cm thick, 114 gram / per patty) in the USDA-inspected Meat Laboratory at Iowa State University (ISU). The fat content of the ground beef patties was determined with an Anyl-Ray Fat Analyzer (Kartridg Pak Co. Davenport, IA, U.S.A.), and was 19%-25%. Beef patties for both experiment 1 and 2 were manufactured using a Hollymatic (Model 54) patty machine (Hollymatic Corporation, Countryside, IL, U.S.A). Single patties were placed into high barrier pouches (Curlon Grade 861, 3cc O_2 / 645 cm^2 / 24 h at 23 ºC and 0% RH; Cryovac Division, W.R. Grace Co., Duncan, SC, U.S.A.) for packaging. The patties for inoculation were immediately transferred to the Pathogen Laboratory in the Iowa State University Food Safety Research Laboratory (ISU-FSRL).

Preparation of bacterial cultures

Five strains of *E. coli O157:H7* were used to make a cocktail to represent different serotypes of this pathogen. These cultures were supplied by the ISU-FSRL and

included ATCC 35150, ATCC 43894, ATCC 43895, WS 3062, and WS 3331. Frozen stocks were separately transferred to 10 ml Tryptic Soy Broth (TSB) (Difco, Detroit, MI, U.S.A.) and incubated at 35 ºC for 24 hours. The cultures were streaked onto MacConkey Sorbitol (SMAC) Agar (Difco) plates and incubated at 37 ºC for 24 hours. The characteristic colonies from SMAC plates were inoculated into TSB and incubated at 35 ºC for 24 hours. One milliliter of each culture was then transferred into 99 ml TSB and incubated at 35 ºC for 24 hours. The concentration of the bacteria reached about 8 log /ml. The inoculum was prepared by adding 2 ml of each culture to 90 ml of peptone water. The cocktail contained approximately equal numbers of each strain with a total concentration of bacteria of 7 log cfu /ml.

Inoculation and packaging

One milliliter of inoculum was placed on the beef patty in each pouch with a sterilized pipette. The packages were manually massaged for about 1-2 min to distribute the inoculum evenly. The concentration of the bacteria on each patty was approximately 5 log /gram. Pouches were immediately vacuum or MAP packaged with a Multivac (model A 300/52) packaging machine (Multivac Inc, Wolfertschwenden, Germany) in the FSRL. Cylinders with the desired gas mixture (99.5% CO_2 and 0.5% CO) for MAP packaging were purchased from Linweld Co. (Linweld Co., Lincoln, NE, U.S.A.). The MAP packaging was done by first applying vacuum (10-13 mbars), then flushing the gas mixture into the pouches (pressure of 680-700 bars) with simultaneous sealing. The volume ratio of gas to the beef patty in a single MAP package was about 4:1. After inoculation and packaging, samples were stored at 2-4 ºC for 12 hours before irradiation.

Irradiation

The inoculated, packaged samples were irradiated at the Iowa State University Linear Accelerator Facility (ISU-LAF). The irradiation was generated by a Circe-III linear electron accelerator at an energy level of 10 MeV and 10 kW (MeV Industries S.A., Jouy-Josas, Cedex, France). The target irradiation doses were 0.5, 1.0 and 1.5 kGy. Alanine pellet dosimeters (5 mm × 5 mm) (Broker Analytische Messtechnik, Rheinstetten, Germany) were placed on the top and bottom surfaces of sample pouches to measure the actual absorbed energy (dose). Immediately after irradiation, the absorbed doses were measured by electron paramagnetic resonance on a Broker EMS 104 EPR Analyzer. The average surface dose, overall average dose and average maximum doses absorbed by the beef patties in vacuum and MAP are listed in Table 1.

Following irradiation, the samples were stored at 2-4 ºC in the FSRL Pathogen Laboratory.

Enumeration

Determination of D_{10}-values: Plating was conducted immediately after the irradiation. Whole patties were massaged manually from outside of the pouches. Then, twenty-five grams of sample from each package was aseptically weighed into a sterile plastic stomacher bag (Whirl-Pack filter bag B01318, Nasco, Fort Atkinson, WI, U.S.A.) with 225 ml of peptone water (Difco, Becton Dickinson, Sparks, MD, U.S.A.), homogenized in a Stomacher blender (Seward Stomacher Blender, Model 4000, Tekmar Co., Cincinnati, OH, U.S.A.) for 60 seconds with high speed, and surface plated onto Sorbitol MacConkey agar (Difco) containing 1 ppm novobiocin and 0.8 ppm potassium

tellurite (Sigma Chemical Co., St. Louis, MO, U.S.A.). The plates were incubated at 37 ºC for 24 hours before counting.

Irradiation D_{10}-value is defined as the amount of radiation energy (dose) needed to reduce 90% (cfu) of the microorganisms in irradiated food products (Thayer and Boyd 1993). The D_{10}-value was determined in this study by calculating the negative reciprocal of the slope of the regression line of the plot of log number of survivors ($\log_{10}$ cfu / gram) versus irradiation dose (kGy) (Chirinos and others 2002).

Enumeration of survivors during storage: Recovery of the pathogen in irradiated ground beef was measured following irradiation and after 24 and 48 hours of storage at 2-4 ºC, and at one week intervals for six weeks to determine the fate of the survivors. For the temperature abuse test, samples were held for 7 days at 2-4 ºC followed by room temperature (25 ºC) for 48 hours prior to enumeration. The plating method was the same as for determination of D_{-10} values.

Experiment 2: *Experiment 2 was designed to determine and compare quality and sensory attributes of irradiated ground beef patties packaged with vacuum or with high CO_2 + CO MAP packaging.*

Packaging of meat samples

Uninoculated patties (see experiment 1) were packaged in the ISU Meat Laboratory using a Multivac (model C500) packaging machine (Multivac Inc, Wolfertschwenden, Germany). The vacuum and gas packaging procedures were the same as in experiment 1.

Irradiation

Uninoculated samples were irradiated at the same facility (ISU-LAF) but at a different time than the inoculated samples. The target doses for experiment 2 were 1.0 kGy and 1.5 kGy. The average surface dose, overall average dose and average maximum doses absorbed by the beef patties in vacuum and MAP were measured as described earlier and are listed in table 2.

Following irradiation, the samples were stored at 2-4 ºC prior to quality evaluation. The samples for sensory evaluation were transferred to the ISU Sensory Evaluation Center immediately following irradiation.

Color Measurement

CIE color values (L*, a* b*) on the surface of the beef patties were measured with a Hunter Lab LabScan (Model LS 1500, Hunter Associated Labratories Inc., Reston, VA, U.S.A.). CIE standard illuminant A (incandescent or tungsten lamplight), 10 degree observer and a 1.75-inch port insert were used. The temperature of the light source was 2,856 ºK. Color of the beef patties was measured on the packaged samples through the packaging material without opening the packages. Three readings were randomly collected from different locations on each sample. Measurements were conducted on day 1 and day 7 after irradiation.

Package Purge

After the color measurement, the samples used to assess color were also used for purge measurement. Purge was measured by weighing the empty pouches before packaging, then weighing the packaged sample with packaging material prior to opening

the package. The sample was then removed, dried with paper towels and weighed. The quantity of purge was determined by subtracting the weight of the packaging film and the weight of the irradiated samples removed from the packages from the weight of the packaged sample before it was opened.

Sample pH

The pH of the samples was measured with a FC 200B pH electrode (Hanna Corporation, Hanna USA, www.hannainst.com) at 25 ºC immediately following the purge measurement by direct insertion of the electrode into the samples.

Oxidative rancidity

Oxidative status of the patties was assessed using the 2-thiobarbituric acid (TBA) distillation procedure (Tarladgis and others 1960). Absorbance of the chromophore produced by the reaction between 2-thiobarbituric acid and malonaldehyde (one of the lipid oxidation products) at 532 nm was automatically converted to mg of malonaldehyde per kg of sample by a computerized Beckman Du 640 spectrophotometer (Beckman Coulter, Canada). Duplicate TBA values per sample were measured and recorded.

Sensory evaluation

Sensory evaluation of raw and cooked hamburger patties was conducted using a ten-member sensory panel of faculty, staff, and students at Iowa State University. All panelists were volunteers and the project was approved by the Iowa State University Human Subjects Review Committee. Panelists were trained to evaluate the sensory attributes in two one-hour training sessions. Each panelist evaluated six samples per

session. Three sessions were conducted each for raw and for cooked beef patties. A computerized sensory scoring system (COMPUSENSE five, v 4.4, Compusense, Inc. Guelph, Ontario, Canada N1H3N4) was used to collect sensory evaluation data.

Raw Hamburger Patties*:* Raw patties were evaluated for color and aroma. For the aroma evaluation, individually wrapped cold (4 ºC) samples labeled with random three-digit codes were simultaneously presented to the panelists in randomized order on pre-cooled trays. Panelists were instructed to cut open the bag as close to the sample as possible, wait 3-5 seconds, and smell the sample. Testing was conducted in partitioned booths and under red fluorescent lights. Samples were evaluated for off-aroma (irradiated); sour-like aroma and ground beef aroma. A 15-unit line scale was used for each attribute with descriptive anchors (left anchor-none, right anchor-intense) at each end of the line.

For color evaluation, the refrigerated patties, on white ceramic plates and in their original packages, were labeled with random three digit codes, and placed on a white paper background for panelists to evaluate the color of a single hamburger patty from each treatment. The patties were evaluated under white florescent lighting positioned to provide 70 foot-candles at the counter surface. Panelists evaluated the intensity of pink / red color. A line scale with 15-units of numerical value was used to collect the data. The left anchor represented none and the right anchor represented intense.

Cooked Hamburger Patties*:* Cooked patties were evaluated for aroma, texture, and flavor. Patties were grilled on a George Foreman Indoor/Outdoor Grill (Model GGR62, Lake Forest, IL, U.S.A.) to an internal temperature of 72 ºC. The temperature of the patties was monitored using a thermocouple (Chromega/Alomega) attached to an Omega digital thermometer (Model DSS-650, Omega Engineering). Two patties per

treatment were prepared and panelists received one-sixth of a patty in a covered, 4-ounce Styrofoam container labeled with a random three-digit code. Cooking was in randomized order with samples presented sequentially immediately after cutting. Testing was conducted in partitioned booths and under red fluorescent lights. A 15-unit line scale with descriptors representing low intensity (none) at the left and high intensity (intense) at the right was used for scoring the following attributes: off-aroma (irradiated), sour-like aroma, ground beef aroma, off-flavor (irradiated), sourness, and ground beef flavor. Juiciness (left anchor-not juicy, right anchor-very juicy) was also evaluated.

Statistical Analysis

A general linear model (SPSS 14.0 Window Grad Pack) was used to evaluate the effects of irradiation dose, packaging types and storage time. When there were significant effects or interactions ($p<0.05$) between experimental factors, linear contrast test, independent sample T-test or post-hoc tests of differences with Tukey adjustment were used to determine the significance of main and simple main effects, or simple-simple main effects.

A mixed linear model was fit with PROC MIXED (SAS Inst., Inc., Cary, N.C., U.S.A, version 9.1) to determine the effects of irradiation dose and packaging technique on the sensory attributes. A random subject term was fitted to incorporate subject-to-subject variability. When a fixed effect was significant ($p<0.05$), post-hoc tests of differences were calculated and then adjusted with the Tukey procedure.

RESULTS AND DISCUSSION

Experiment 1

Radiation D_{10}-values

Table 3 shows that there was no statistically significant difference between radiation D_{10}-value for *E. coli O15:H7* on irradiated ground beef patties packaged in vacuum or in high CO_2 MAP packages (p value: 0.315). The mean radiation D_{10}-value of this pathogen in MAP was slightly higher (0.50 ± 0.02 kGy) than in vacuum (0.47 ± 0.02 kGy), but there were no significant effects of packaging type, irradiation dose, or interaction between the experimental factors (ANOVA not shown). In USDA-proposed rules (1999), the recommended radiation D_{10}-value for *E. coli O157:H7* in ground beef at refrigeration temperature was 0.25 kGy. There was no specification of packaging type. Chiasson and others (2005) observed a much lower radiation D_{10}-value for *E. coli O157:H7* in sterilized vacuum-packaged ground beef (0.118 kGy) or in 100% CO_2 MAP package (0.123 kGy) after the samples were exposed to 0.1 or 0.6 kGy gamma irradiation. Thayer and Boyd (2001) reported that the D_{10}-value of this organism on vacuum packaged sterilized ground beef was 0.39 ± 0.04 kGy, which is also lower than our result. Thayer and Boyd (1993) previously reported the D_{10}-value of this pathogen on vacuum packaged sterilized ground beef as 0.27 ± 0.03 kGy when *E. coli O157:H7* was in stationary growth phase. Fu and others (1995) observed a 2 log reduction of this pathogen at 0.8 kGy (D_{10}-value was about 0.4 kGy) in aerobic packaged ground beef at refrigeration temperature. In a study by Clavero and others (1994), beef patties inoculated with a five-strain cocktail of *E. coli O157:H7* were exposed to gamma irradiation ranging from 0.5 to 3.0 kGy. The D_{10}-value from this study was reported as 0.241 kGy. Chirinos

and others (2002) reported that the D_{10}-value for this pathogen was from 0.17 kGy to 0.27 kGy in hamburgers wrapped in polyvinyl chloride film and irradiated at 3.0 and 5.0 kGy. Some studies demonstrated that bacteria were more sensitive to irradiation under aerobic than anaerobic condition; therefore, it might be expected that radicals and ozone formed by irradiation in the presence of oxygen would increase bacteria reduction rates (Lee and others 1996; Chiasson and others 2005). However, Patteson's study (1988) reported that D_{10}-value for *E. coli* was lower in 100% CO_2 (0.288 kGy) and vacuum (0.271 kGy) packaging than in air (0.351 kGy). The D_{10}-value in 100% CO_2 was not significantly different from vacuum. The authors suggested that *E. coli* was more sensitive to irradiation in high CO_2 MAP than in aerobic packaging, and there was synergistic interaction between irradiation and CO_2 to enhance the pathogen reduction. In the present study, 0.5% CO included in MAP had no effect on the pathogen reduction rate by irradiation. Gee and Brown (1980) also indicated that CO at levels below 1% in MAP had a negligible effect on the microbial characteristics.

In general, the environmental factors that affect the radiation sensitivity of pathogens include the composition of the product (matrices), temperature, bacterial strains, pH, packaging type, available water, the growth phase of bacteria, and plating techniques (Thayer and Boyd 1993; Lee and others 1996; Buchanan and others 1999; Dickson 2001; Thayer and Boyd 2001, Jordan and Maher 2005). However, in the studies for the determination of the radiation sensitivity of this pathogen, different *E. coli* stains and different plating techniques were used, and some of the reports did not include the pH of the products. The product matrices used in those studies were also different, ranging from chicken meat to ground beef. Some studies used fresh refrigerated products, while other studies used sterilized or frozen products that were thawed to refrigeration

temperature. It can be expected that frozen ground beef and frozen chicken meat will result in greater purge during thawing. The purge could provide more available water to produce hydroxyl radicals and hydrogen peroxide during irradiation, which could enhance the pathogen reduction rate and decrease D_{10}-value. Consequently, there are many factors that may contribute to differences in the reported D_{10}-values.

Recovery of *E. coli O157:H7*

The bacterial counts following irradiation (day 1) were compared with the counts after 24 hours (day 2) and 48 hours (day 3) at 2-4 ºC to determine the recovery of the survivors at refrigeration temperature. The results presented in table 4 (ANOVA) and table 5 (results from three replicated experiments) showed that there was no statistically significant growth after 24 or 48 hours of storage (p-value: 0.800) at refrigeration temperature irrespective of the packaging type (p-value: 0.532). The recovery data from this study were compared with the survival curve of the broth-based anaerobic growth model for *E. coli O157:H7* in the USDA Pathogen Modeling Program (PMA, version 7.0). Using the parameters of 5.0 ºC (lowest temperature in the model), pH 5.7 (average pH for ground beef) and salt concentration of 0.5% (the lowest concentration in the model) for predicting the pathogen growth in irradiated ground beef patties packaged in vacuum and high CO_2 MAP packages during 24 or 48 hour storage, resulted in no significant growth predicted by the model under these conditions. In the growth models developed by Buchanan and Bagi (1994), and in the report of Rajkowski and Marmer (1995), the minimum growth temperature for *E. coli O157:H7* in optimal conditions (in BHI broth) was 8-10 ºC. Mann and Brashears (2005) reported that there was no significant growth of *E. coli O157:H7* (a three strain cocktail mixture) in ground beef (packaged in sterilized whirl-pack bags) stored at 4.4-7.2 ºC for 24 or 48 hours. Li and

Logue (2005) also reported no growth of this organism in minced bison meat during the storage at 5 ºC.

The survival of *E. coli O157:H7* during refrigerated storage

Table 6 shows the overall results of analysis of variance for survival of *E. coli O157:H7* in irradiated ground beef patties packaged in vacuum or MAP for 6 weeks. There were significant effects of storage times (p-value: 0.001), package techniques (p-value: 0.028) and irradiation dose (p-value: 0.000). However, there were also three way interactions between irradiation dose, packaging type and replications, and four way interactions between irradiation dose, storage week, packaging type and replications. Therefore, the data for each replication were analyzed separately and the results are presented in tables 7-12.

Replicate 1: Tables 7 and 8 show that the cell numbers of *E. coli O157:H7* in ground beef patties in control-vacuum (without irradiation) packages did not change significantly through 6 weeks of post-irradiation storage at 4 ºC. The pathogen in control-MAP packages also remained the same except for a decrease from week 5 to week 6. In the irradiated vacuum packages, the cell numbers decreased significantly in 1.0 kGy and 1.5 kGy-vacuum packages after week 3, and after week 4 in 0.5 kGy-vacuum packages. The counts of *E. coli O157:H7* in all irradiated MAP packages survived through 6 week without significant change.

Replicate 2: The counts of *E. coli O157:H7* in control-vacuum packages decreased significantly from week 5 to week 6 (tables 9 and 10). There were increased counts in control-MAP packages from week 2 to week 3, but the counts were then reduced from week 3 to week 4. The ultimate cell counts were not significantly different from week 1 to week 6. The pathogen numbers in the 0.5 kGy and 1.0 kGy-vacuum

packages decreased after week 3 or week 4, respectively. Although the results showed no significant changes in the 1.5 kGy-vacuum packages, the plating method used for the experiment could not detect the pathogen in some of the 1.5 kGy-packages at week 6. The pathogen reduction in the 1.0 kGy-vacuum packages was 0.45-2.8 logs more than in the 1.0 kGy-MAP packages, and the reduction in the 1.5 kGy-vacuum packages was 0.28-3.27 logs more than in the 1.5 kGy-MAP packages.

Replicate 3: As in replicate 2, *E. coli O157:H7* in vacuum control packages was reduced after 4 to 5 weeks of storage ((tables 11 and 12). The cell counts in control-MAP packages gradually decreased from week 3 to week 6. The pathogen in the 0.5 kGy-vacuum packages was reduced after week 5, but there was no significant change in the 1.0 kGy-vacuum packages through 6 weeks. In the 1.5 kGy-vacuum packages, cells could not be detected in some of the packages after 6 weeks of storage. In the irradiated MAP packages, counts in the 0.5 kGy-packages decreased from week 3 to week 6. Cell counts in the 1.0 kGy packages were reduced from week 4 to week 6, while in the 1.5 kGy-MAP packages, the reduction in cell counts began after week 2.

The overall results from the three experimental replications showed that *E. coli O157:H7* on irradiated or non-irradiated beef patties can survive, though not grow, in most vacuum and high CO_2 MAP packages for at least three weeks before decreasing in numbers. Since the interactions between packaging, dose and storage were not consistent in the three replications, we could only conclude that there was little packaging effect on the survival of *E. coli O157:H7* at refrigeration temperature. There was no lag phase observed for *E. coli O157:H7* in any of the packages during the storage period at 4 ºC, similar to observations made by Dickson and Olson (2001). The number of survivors gradually deceased during the storage period for both irradiated and non-irradiated beef

patties in most of the packages after 3 or 4 weeks. This result was similar to the study by Dykes and others (2001). These authors reported that the numbers of *E. coli O157:H7* on beef steaks did not change significantly in vacuum or 100% CO_2 MAP during 2 weeks of storage at 4 ºC. Uyttendaele and others (2001) also reported that vacuum or MAP (40% CO_2 and 60% N_2) packaging did not significantly affect the survival of *E. coli O157:H7* on sliced beef during storage at 4ºC for 28 days, although there were some reductions observed in MAP or vacuum packages after 7 days. However, Badr (2005) reported that *E. coli O157:H7* on beef sausages irradiated at 1.0 or 2.0 kGy increased in number significantly after 12 days of storage at 4 ºC. After studying of the fate of six strains of *E. coli O157:H7* on ground beef at different storage temperatures, Barkocy-Gallagher and others (2002) concluded that temperatures of 4 ºC or below can limit the growth of this pathogen, but barely affect the survival. In the model developed by Tamplin and others (2005), *E. coli O157:H7* in sterilized ground beef can grow at 6 ºC, although it was observed from the growth curve that this temperature was a critical temperature for the growth of this pathogen. Since temperature of 4 °C or below is not physiologically feasible for *E. coli O157:H7* growth, the inconsistent counts in some vacuum or MAP packages in the present study were treated as variations in the enumeration. Least significant difference (LSD) has been used in many other studies for the multiple comparisons of bacteria counts with time, and a greater number of effects can often be observed using this statistical method. However, the Tukey adjustment was used in the present study for the multiple comparisons. Because the significant effects shown with this method are less than with LSD, it is considered a more conservative approach for evaluation of the growth or survival of food-borne pathogens.

The beef patties used in this study was a simulation of commercial products. A sour odor was observed in both vacuum and MAP packages after three to four weeks of storage. Although the level of background flora was not tested, it can be expected to contribute to variations of the bacteria counts and may have affected the pathogen growth during long term storage. Many studies have demonstrated the rapid growth of lactic acid bacteria on unirradiated fresh meat products in 100% CO_2 MAP or in vacuum packaging after 3 or 4 weeks of storage at refrigeration temperature (Gill and Harrison 1989; Jackson and others 1992; Tewari and others 1999). Bredholt and others (1999) demonstrated in their study that the indigenous lactic acid bacteria could inhibit the growth of both *E. coli O157:H7* and *Listeria monocytogenes* in vacuum or MAP-packaged cooked meat. However, Uyttendaele and others (2001) reported that the lactic acid bacteria growing in vacuum or MAP packaging did not affect the survival of *E. coli O157:H7* on beef tissue. Dickson and Olson (2001) also indicated that the background microflora would not affect the outgrowth of *E. coli O157:H7* in irradiated ground beef.

The growth of *E. coli O157:H7* during temperature abuse

After 7 days of storage at 4 ºC, a group of vacuum and MAP packages with irradiated ground beef were placed at room temperature (25 °C) for 48 hours. Table 13 (ANOVA) shows that the temperature effect was significant (p-value: 0.007). There were interactions between dose, temperature, packaging and replications (p-value: 0.001), therefore, the results from each replication are presented in tables 14, 15 and 16. In replicate 1, the numbers of *E. coli O157:H7* increased significantly only in the 1.0 kGy-vacuum packages. In replicate 2, the bacteria increased significantly in 0.5 kGy and 1.5 kGy-vacuum packages. In replicate 3, the population of this microorganism increased significantly only in 1.5 kGy-MAP packages. Although *E. coli O157:H7* multiplied in

some of samples mentioned above under temperature abuse, the cell number only reached 3-5 log cfu /g after 48 hours. According to the growth model by the USDA Pathogen Modeling Program, *E. coli O157:H7* can grow to the stationary phase (8 log cfu /g) in broth culture (pH 6.0, 0.5 % sodium chloride) after 25 hours of exposure to 25 ºC. Fu and others (1995) reported that after 7 days of storage at 7 ºC, rapid growth of *E. coli O157:H7* in irradiated ground beef occurred during 2 days of temperature abuse at 25 ºC regardless of packaging (vacuum or air) or irradiation dose (0.80 or 2.0 kGy). Thayer and Boyd (1993) observed the growth of *E. coli O157:H7* on sterilized ground beef (irradiated at 0.75 kGy) in vacuum packages during 20 hours of temperature abuse at 35 ºC, however, this was observed for only one replication out of three. In the same study, the pathogen on unirradiated samples grew rapidly to a much higher level than the initial inoculated concentration (about 4 logs). Some studies of temperature abuse have involved exposure of *E. coli O157:H7* to temperature abuse immediately after irradiation or packaging (Gill and Delacy 1991; Thayer and Boyd 1993; Tamplin and others 2005). In the study of Fu and others (1995), the samples were stored at 7 ºC for 1 week before exposure to room temperature. This storage temperature was higher than the critical growth temperature predicted by Tamplin and others (2005). However, the samples in the present study were stored at refrigeration temperature (4 ºC) for 7 days before the temperature abuse. The growth of the pathogen in this study involved a greater temperature shift. In the model developed by Zwietering and others (1994) (using *Lactobacillus plantarum*), it was predicted that shifts in temperature from close to the minimum growth temperature to a higher temperature can cause bacterial death, or may cause a large variation of prediction. Therefore, relative to the safety of meat products, further study is needed for predicting the growth of *E. coli O157:H7* with different

temperature fluctuations during storage or transportation, including the effects of shifts from refrigeration temperature to possible product abuse temperatures.

In summary of the results from experiment 1, the radiation sensitivity of *E. coli O157:H7* in ground beef was similar in both vacuum and high CO_2 MAP. High CO_2 MAP did not further eliminate the pathogen during post-irradiation storage at 4 °C; therefore, if irradiation can not completely eliminate the pathogen at initial stages, the survivors are likely to remain viable for at least 6 weeks at refrigeration temperature in both vacuum and high CO_2 MAP packages. Further, *E. coli O157:H7* survivors may be able to grow in irradiated beef patties packaged in vacuum or high CO_2 MAP during temperature abuse, although the growth rate might be slower in comparison of what is predicted by USDA Pathogen Modeling Program.

Experiment 2

Color values (L* a*b*)

Tables 17, 18 and 19 show the results of color measurements from three replications. The a* value represents redness (positive value) or greenness (negative value). Beef patties in MAP packages were significantly redder than in vacuum packages from day 1 to day 7 regardless of irradiation treatment. This result was consistent with the observation reported by Kusmider and others (2002). It also confirmed other reports regarding fresh meat color in MAP + CO packages (Sorheim and others 1999; Luno and others 2000; Krause and others 2003; Viana and others 2005). All samples in MAP packages were also more yellow (greater b* value) than samples in vacuum packages (table 19). Huffman and others (1984) observed that carbon monoxide increased a value and b value of beef steaks, but carbon dioxide decreased the b value. However, Berruga

and others (2005b) reported that high concentration of CO_2 (80%) in MAP increased b* value of fresh lamb meat. For the L* value in the present study, all samples in vacuum packages were lighter (greater L* value) than in MAP packages irrespective to the irradiation doses or storage time. This result is different from the observations of Kusmider and others (2002) in a similar study, who reported that beef patties in MAP packages had greater L* value than those in vacuum packages.

Package Purge and pH

The overall result for package purge (ANOVA not shown) indicated no significant effects of packaging types, irradiation doses or storage time, nor interaction between any of the treatment factors. Table 20 shows that the purge in all irradiated vacuum packages was significantly more on day 7 than in irradiated MAP packages. However, the variation in the amount of purge in MAP packages was much smaller than in vacuum packages. Therefore, using ANOVA to test the hypothesis (significant difference between groups) was not feasible, because the analysis of variance (ANOVA) is based on the assumption of equal variance. Unequal variance in independent sample T-tests showed that in replication 1, there was significantly more purge in the 1.5 kGy-vacuum packages than in the 1.5 kGy-MAP packages after 7 days of storage. In replication 2, there was significantly more purge in the1.0 kGy-vacuum packages than in the 1.0 kGy-MAP on day 1. In replication 3, there was also significantly more purge in the 1.0 kGy-vacuum packages than in the 1.0 kGy-MAP packages on day 1, and there was significantly more purge in the 1.5 kGy-vacuum packages than in the 1.5 kGy-MAP packages on day 7. However, in the study by Krause and others (2003), the purge from pork loins in MAP (70% CO_2, 29.5 % N_2 and 0.5% CO) was more than in vacuum packages. Purge is often related to meat pH, with lower pH resulting in greater purge.

However, in the present study, the pH of beef patties was not affected no by packaging, irradiation dose or storage time (table 21). Therefore, we cannot relate the product pH to package purge. Sorheim and others (2004) indicated that the pH of the ground beef in 100% CO_2 MAP packages was 0.12 lower than in vacuum packages; therefore, these authors suggested that the lower pH might be one reason for high cooking loss in ground beef packaged in high CO_2 MAP. Martinez and others (2005) also observed that the pH of pork sausages in MAP packages was decreased when the concentration of CO_2 in the packages was increased. These authors reported that the pH of the pork sausages was 5.25 in the MAP with 60% CO_2. However, Huffman and others (1984) and Holley and others (1994) reported that 100% CO_2 in MAP packaging for restructured beef steaks or pork loins did not have significant pH effect. Vergara and others (2005) reported that the pH of the rabbit meat packaged in 80% CO_2 + 20% O_2 MAP packaging was the same as the rabbit meat packaged in 30% or 40% CO_2 MAP packages, however, the drip loss and cooking loss were greater for the rabbit meat in higher CO_2 packages. Jackobsen and Bertelsen (2002, 2004) indicated that a large amount of CO_2 absorbed (dissolution) in the meat product can decrease the surface pH of the meat product slightly. However, the small pH delineation may not be detected if whole muscle is used for the analysis (Holley and others 1994).

Oxidation rancidity

Overall ANOVA (table 22) for TBA values of the ground beef patties showed no significant irradiation dose effect or storage effect, but there was a significant packaging effect (p-value: 0.04). There were also interactions between storage, dose and replication (p-value: 0.016) and between packaging, dose and replication (p-value: 0.04). Therefore, data for single replications were again separately analyzed. Table 23 shows the TBA

values of irradiated ground beef patties in vacuum or MAP packages on day 1 and day 7 in three experimental replications. After 7 days of storage, the TBA value was higher in the control group of both vacuum and MAP treatments, and also higher in the 1.0 kGy and 1.5 kGy-MAP packages. Although statistical analysis showed significantly higher TBA value in some MAP and vacuum packages, the TBA value in all treatment packages were well below 1.0, which is often considered the threshold level for rancidity (Mattison and others 1986). Further, the results in replication 2 showed that the TBA value in vacuum control and MAP control treatments were different from day 1 to day 7, which suggests that the baseline TBA for the two packaging types were different before the irradiation treatment. The reason for this difference is not clear. John and others (2005) also reported that case-ready fresh beef had higher TBA values in low oxygen MAP with 0.4% CO (0.7 ± 0.1) than in vacuum packages (0.4 ± 0.0); however, both TBA values were below the rancidity threshold level (1.0). Krause and others (2003) reported that the TBA values of pork chops were not significantly different in vacuum packages compared to low oxygen MAP + CO packages. One of the pathways of irradiation-accelerated lipid oxidation is through existing oxygen in the product environment to produce high oxidative compounds, such as hydroperoxides (Lefebvre and others 1994; Nawar 1996). Luchsinger and others (1996b) noticed that pork chops irradiated in vacuum packages exhibited much lower lipid oxidation than pork chops irradiated in aerobic bags. Lefebvre and others (1994) and Ahn and others (2000) suggested that lipid oxidation caused by irradiation could be reduced by vacuum or modified atmosphere packaging. From the stand point of preventing lipid oxidation induced by irradiation, it appears that low oxygen MAP is as useful as vacuum packaging.

Sensory evaluation

Tables 24-28 show the sensory evaluation results for raw and cooked irradiated ground beef patties from vacuum and MAP packages. For raw beef patties, the sensory attributes evaluated were red color, irradiation off-aroma, sour-like aroma and raw ground beef aroma. For the raw patties, intensity of red color in MAP was rated much higher than for vacuum packages irrespective of irradiation doses. This is similar to the color value measured by the Hunter LAB LabScan. Irradiated beef patties had significantly more intense off-aroma than unirradiated beef patties regardless of packaging type; however, the intensity was similar for packages irradiated at 1.0 kGy and 1.5 kGy. Kusmider and others (2002) also reported no significant difference for this attribute for irradiated beef patties packaged by vacuum and MAP immediately after irradiation; although the authors reported that the off-odor was much lower in MAP packaging than in vacuum after 28 days of storage. Comments from the panelists indicated that the off-aroma was more intense when irradiated MAP packages were first opened, but that after the off-aroma dissipated, the beef patties from irradiated MAP packages smelled better than the patties from irradiated vacuum packages. This is an important observation for irradiated meat products in MAP packaging, because when the package headspace gas mixed with volatiles produced by irradiation was directly inhaled by panelists, the off-odor appeared to be much stronger than when smelling the product surface after opening the packages. There was no significant difference for sour-like aroma in either packaging treatment. Although there was statistically more beef aroma in irradiated beef patties from vacuum packages than from MAP packages, and more beef aroma in unirradiated beef patties than irradiated beef patties, the differences were small (1 unit) on 15 unit line scale.

For cooked ground beef patties, 7 sensory attributes were evaluated, including ground beef aroma, ground beef flavor, irradiation off-aroma, irradiation off-flavor, juiciness, sour-like aroma and sourness. Overall results showed that there was no significant difference of sour-like aroma, juiciness or sourness in beef patties, irrespective of packaging type or irradiation dose. Unirradiated beef patties had slightly more intense ground beef aroma than irradiated patties. There was more irradiated off-aroma and off-flavor, however, less ground beef aroma and flavor in cooked beef patties from irradiated MAP packages than from irradiated vacuum packages.

Irradiation off-aroma (irradiated off-odor) has been well-studied (Luchsinger and others 1997; Montgomery and others 2000; Wheeler and others 1999; Lee and Ahn 2003). The radiolytic volatiles, such as sulfur and carbonyl compounds, produced by irradiation, are major contributors to the off-odor in irradiated vacuum packaged fresh meat (Kim and others 2002). In the present study, the sour-like aroma or sourness in beef patties packaged in high CO_2 MAP was similar to vacuum packaged patties, although some reports have indicated that high concentration of CO_2 in MAP caused a sour taste of ground beef ((Jakobsen and Bertelsen 2002; Sorheim and others 2004).

CONCLUSIONS

The present study demonstrated that irradiation was effective to reduce *E. coli O157:H7* in ground beef. Irradiation with the dose of 1.5 kGy was able to reduce 3 log cfu /g of this pathogen in ground beef patties packaged in vacuum or high CO_2 MAP. High CO_2 MAP was not superior to vacuum for control of this pathogen in ground beef either alone or in combination with irradiation, as far as the survival of this pathogen was

concerned during short term or long term storage at refrigeration temperature or with temperature abuse. An advantage of high CO_2 MAP + CO was the retention of bright red fresh meat color that is normally affected by irradiation or vacuum packaging. The other quality measurements including package purge, product pH and oxidative rancidity were similar for the products packaged in either vacuum or MAP packaging. A disadvantage of high CO_2 MAP, however, appeared to be somewhat greater irradiated off-odor in MAP-patties relative to vacuum. Consequently, it remains important to minimize the irradiated off-odor that may develop in ground beef patties from irradiation, if high CO_2 MAP + CO packaging is to be used effectively for its color advantage in irradiated beef patties.

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Table 1--Absorbed irradiation doses by ground beef patties packaged in vacuum and high CO_2 MAP + CO (experiment 1)

Target doses (kGy)	Average surface dose (kGy)	Average maximum dose (kGy)	Overall average dose (kGy)
0.5 kGy	0.51 kGy	0.61 kGy	0.56 kGy
1.0 kGy	1.03 kGy	1.25 kGy	1.14 kGy
1.5 kGy	1.49 kGy	1.81 kGy	1.65 kGy

Table 2--Absorbed irradiation doses by ground beef patties packaged in vacuum and high CO_2 MAP + CO (experiment 2)

Target doses (kGy)	Average surface dose (kGy)	Average maximum dose (kGy)	Overall average dose (kGy)
1.0 kGy	1.01 kGy	1.24 kGy	1.12 kGy
1.5 kGy	1.52 kGy	1.82 kGy	1.67 kGy

Table 3—Means of radiation D_{10}-values (kGy) for *E. coli O157: H7* on ground beef patties in vacuum or in high CO_2 MAP packages

Packaging	N	Mean D_{10}-Value	Std. Deviation	Std. Error Mean	p-value
Vacuum	9	0.45	0.09	0.02	0.32
MAP	9	0.50	0.08	0.02	

Table 4--Analysis of variance: Recovery of *E. coli O157:H7* on irradiated beef patties in vacuum or MAP packages after two days of storage at 2-4ºC

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	3478.115	3047.967	0.000	0.999
Pack	1	0.723	0.562	0.532	0.219
Dose	3	97.564	239.610	0.000	0.992
Time	2	0.085	0.236	0.800	0.106
Rep	2	1.141	0.766	0.553	0.389
Pack * Dose	3	0.107	0.767	0.553	0.277
Pack * Time	2	0.080	0.317	0.745	0.137
Dose * Time	6	0.109	0.288	0.932	0.126
Pack * Dose * Time	6	0.238	1.145	0.395	0.364
Pack * Rep	2	1.287	7.018	0.176	0.905
Dose * Rep	6	0.407	1.310	0.390	0.606
Pack * Dose * Rep	6	0.139	0.667	0.678	0.250
Time * Rep	4	0.359	0.846	0.546	0.372
Pack * Time * Rep	4	0.253	1.214	0.355	0.288
Dose * Time * Rep	12	0.380	1.825	0.155	0.646
Pack * Dose * Time * Rep	12	0.208	1.392	0.176	0.104

Table 5—The recovery of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties packaged in vacuum or MAP after 24 or 48 hours of storage at 2-4ºC

Dose[3] (kGy)	Count (log cfu /g) in vacuum packages[2]						Count (log cfu /g) in MAP packages[2]					
	Day 1 (Irradiation day)	SE[1]	Day 2 (24 hours)	SE[1]	Day 3 (48 hours)	SE[1]	Day 1 (Irradiation day)	SE[1]	Day 2 (24 hours)	SE[1]	Day 3 (48 hours)	SE[1]
0	5.53	0.10	5.70	0.04	5.73	0.13	5.68	0.07	5.67	0.09	5.50	0.06
0.5	4.79	0.10	4.60	0.17	4.59	0.23	4.70	0.13	4.45	0.03	4.48	0.08
1.0	3.51	0.20	3.64	0.04	3.13	0.17	3.47	0.44	3.79	0.08	3.45	0.09
1.5	2.21	0.43	2.42	0.06	2.18	0.56	2.72	0.38	2.62	0.17	2.32	0.43

[1] Standard error of means
[2]No significant difference between the means within each row of the same packaging ($p<0.05$)
[3] The target irradiation dose

Table 6--Analysis of variance: Survival of *E. coli O157:H7* on irradiated beef patties in vacuum or MAP packages during refrigerated storage for 6 weeks

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	4143.526	2583.161	0.000	0.999
Dose	3	292.359	110.838	0.000	0.982
Week	5	8.946	10.101	0.001	0.835
Pack	1	42.620	33.608	0.028	0.944
Rep	2	1.604	0.488	0.641	0.168
Dose * Week	15	0.359	1.223	0.309	0.379
Dose * Pack	3	2.774	2.894	0.124	0.591
Week * Pack	5	1.703	2.702	0.085	0.575
Dose * Week * Pack	15	0.338	0.888	0.583	0.308
Dose * Rep	6	2.638	3.025	0.129	0.793
Week * Rep	10	0.886	1.630	0.280	0.723
Dose * Week * Rep	30	0.294	0.772	0.759	0.436
Pack * Rep	2	1.268	1.049	0.397	0.221
Dose * Pack * Rep	6	0.959	2.520	0.043	0.335
Week * Pack * Rep	10	0.630	1.657	0.138	0.356
Dose * Week * Pack * Rep	30	0.380	2.852	0.000	0.229

Table 7—The survival of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties packaged in vacuum during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.38[a]	0.40	5.25[a]	0.07	4.79[a]	0.03	4.67[a]	0.11	4.75[a]	0.05	4.60[a]	0.06
0.5	3.70[a]	0.09	3.59[a]	0.03	3.25[a,b]	0.30	3.27[a,b]	0.20	2.65[b,c]	0.09	2.33[c]	0.06
1.0	2.06[a]	0.18	1.97[a,c]	0.25	2.03[a]	0.28	1.10[b,c]	0.10	1.78[a,b,c]	0.07	1.12[c]	0.11
1.5	2.04[a]	0.40	1.31[a,b]	0.17	1.31[a,b]	0.21	0.82[b]	0.31	0.30[b]	0.17	0.40[b]	0.20

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 8—The survival of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties packaged in high CO_2 MAP during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.20[a,b]	0.13	5.40[a]	0.05	5.07[a,b]	0.06	5.08[a,b]	0.17	5.36[a]	0.07	4.74[b]	0.07
0.5	4.10[a]	0.12	4.42[a]	0.06	4.06[a]	0.17	4.14[a]	0.11	3.74[a]	0.12	3.94[a]	0.32
1.0	2.78[a]	0.18	2.43[a]	0.12	2.92[a]	0.51	3.11[a]	0.25	3.63[a]	0.04	2.31[a]	0.33
1.5	1.90[a]	0.28	1.62[a]	0.09	2.55[a]	0.32	2.06[a]	0.51	2.69[a]	0.38	1.48[a]	0.14

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of mea
[3]The target irradiation dose

Table 9—The survival of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties packaged in vacuum during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.13[a]	0.06	5.26[a]	0.03	5.10[a]	0.07	5.21[a]	0.03	5.10[a]	0.17	4.14[b]	0.42
0.5	3.66[a]	0.06	4.11[a,b]	0.18	4.46[b]	0.02	4.19[b]	0.06	2.53[c]	0.12	2.71[c]	0.12
1.0	2.41[a]	0.12	1.48[a,b]	0.25	1.79[a,b]	0.49	0.64[b]	0.38	0.46 [b]	0.09	1.10[a,b]	0.10
1.5	1.74[a]	0.18	0.85[a]	0.13	0.87[a]	0.09	1.97[a]	1.02	0.16[a]	0.16	0.10[a]	0.10

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 10—The survival of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties packaged in high CO_2 MAP during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.19[a]	0.01	5.25[a]	0.13	6.23[b]	0.06	5.46[a,b]	0.26	4.75[a]	0.26	4.83[a]	0.20
0.5	4.07[a,b]	0.07	4.36[a]	0.27	4.61[a]	0.12	4.40[a]	0.09	3.45[b,c]	0.07	3.18[b,c]	0.02
1.0	2.25[a]	0.20	2.80[a]	0.22	2.92[a]	0.16	3.03[a]	0.07	2.59[a]	0.23	2.57[a]	0.16
1.5	0.82[a,b]	0.31	1.05[a,b]	0.33	1.72[a]	0.12	1.03[a,b]	0.14	0.62[b]	0.14	0.95[a,b]	0.10

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 11—The survival of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties packaged in vacuum during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log/g)	SE[2]	Mean[1] (log/g)	SE[2]	Mean[1] (log/g)	SE[2]	Mean[1] (log/g)	SE[2]	Mean[1] (log/g)	SE[2]	Mean[1] (log/g)	SE[2]
0	$4.81^{a,c}$	0.01	$5.23^{a,c}$	0.49	5.42^{a}	0.10	$4.86^{a,c}$	0.04	3.55^{b}	0.30	$4.02^{b,c}$	0.21
0.5	$4.12^{a,b}$	0.13	$3.99^{a,b}$	0.82	4.27^{a}	0.08	3.73^{b}	0.04	3.18^{c}	0.08	$3.52^{b,c}$	0.10
1.0	$2.71^{a,b}$	0.22	$2.54^{a,b}$	0.14	2.86^{a}	0.07	$2.03^{a,b}$	0.19	1.85^{b}	0.17	$2.13^{a,b}$	0.29
1.5	$1.64^{a,c}$	0.29	$1.11^{a,b,c}$	0.12	1.99^{a}	0.09	$0.46^{b,c,d}$	0.32	$0.71^{c,d}$	0.39	$0.00^{c,d}$	0.00

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 12—The survival of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties packaged in high CO_2 MAP during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.39[a]	0.08	5.20[a,b,c]	0.10	5.46[a]	0.05	4.84[b,c]	0.16	4.81[c]	0.07	4.22[d]	0.07
0.5	4.45[a]	0.13	4.70[a]	0.06	4.73[a]	0.09	4.08[b]	0.07	4.37[a,b]	0.12	3.54[c]	0.08
1.0	3.35[a]	0.03	3.24[a]	0.05	3.09[a,b]	0.09	2.75[b,c]	0.16	2.84[b,c]	0.05	2.51[c]	0.15
1.5	2.13[a]	0.11	2.08[a]	0.15	1.18[a,b]	0.15	1.31[a,b]	0.17	1.56[a,b]	0.18	0.82[b]	0.42

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 13--Analysis of variance: Growth of *E. coli O157:H7* on irradiated beef patties in vacuum or MAP packages held at 25ºC for 48 hours after 7 days at 4 °C

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	1989.755	769.894	0.001	0.997
Dose	3	56.452	89.067	0.000	0.978
Temp	1	16.376	133.656	0.007	0.985
Pack	1	0.763	0.324	0.627	0.139
Rep	2	2.584	2.182	0.606	0.915
Dose * Temp	3	4.427	4.357	0.060	0.685
Dose * Pack	3	0.494	0.339	0.798	0.145
Temp * Pack	1	0.082	0.682	0.496	0.254
Dose * Temp * Pack	3	0.119	0.177	0.908	0.081
Dose * Rep	6	0.634	0.351	0.884	0.280
Temp * Rep	2	0.123	0.262	0.815	0.378
Dose * Temp * Rep	6	1.016	1.519	0.312	0.603
Pack * Rep	2	2.354	2.586	0.287	0.731
Dose * Pack * Rep	6	1.459	2.181	0.183	0.686
Temp * Pack * Rep	2	0.121	0.180	0.839	0.057
Dose * Temp * Pack * Rep	6	0.669	4.410	0.001	0.216

Table 14—The growth of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties held at 25ºC for 48 hours after 7 days at 4 °C (Rep 1)

Dose[3] (kGy)	Count (log cfu /g) in vacuum packages				Count (log cfu /g) in MAP packages			
	Mean[1] (4 ºC)	SE[2]	Mean[1] (48 hrs at 25ºC)	SE[2]	Mean[1] (4 ºC)	SE[2]	Mean[1] (48 hrs at 25ºC)	SE[2]
0	5.38	0.40	5.69	0.09	5.20	0.13	5.12	0.01
0.5	3.11	0.59	3.66	0.23	4.10	0.12	4.50	0.14
1.0	2.06[a]	0.18	3.58[b]	0.03	2.78	0.18	3.55	0.26
1.5	2.04	0.40	2.76	0.19	2.19	0.08	2.82	0.02

[1]Mean values within the same row of the same packaging type with different superscripts are significantly different ($p<0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 15—The growth of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties held at 25ºC for 48 hours after 7 days at 4 °C (Rep 2)

Dose[3] (kGy)	Count (log cfu /g) in vacuum packages				Count (log cfu /g) in MAP packages			
	Mean[1] (4 ºC)	SE[2]	Mean[1] (48 hrs at 25ºC)	SE[2]	Mean[1] (4 ºC)	SE[2]	Mean[1] (48 hrs at 25ºC)	SE[2]
0	5.13	0.06	5.15	0.04	5.19	0.01	5.16	0.09
0.5	2.82[a]	0.84	4.54[b]	0.07	4.06	0.07	4.51	0.10
1.0	2.41	0.12	2.30	0.20	2.25	0.20	2.73	0.25
1.5	1.74[a]	0.18	4.30[b]	0.37	1.08	0.30	2.16	0.24

[1]Mean values within the same row of the same packaging type with different superscripts are significantly different ($p<0.05$).
[2] Standard error of means
[3]The target irradiation dose

Table 16—The growth of *E. coli O157: H7* (log cfu /gram) in irradiated ground beef patties held at 25ºC for 48 hours after 7 days at 4 °C (Rep 3)

Dose[3] (kGy)	Count (log cfu /g) in vacuum packages				Count (log cfu /g) in MAP packages			
	Mean[1] (4 ºC)	SE[2]	Mean[1] (48 hrs 25ºC)	SE[2]	Mean[1] (4 ºC)	SE[2]	Mean[1] (48 hrs 25ºC)	SE[2]
0	4.81	0.01	5.50	0.20	5.39	0.08	5.46	0.08
0.5	3.68	0.46	4.46	0.13	4.54	0.13	4.23	0.16
1.0	2.71	0.22	2.94	0.64	3.35	0.03	3.50	0.12
1.5	1.64	0.29	2.95	0.25	2.02[a]	0.02	5.29[b]	0.15

[1] Mean values within the same row of the same packaging type with different superscripts are significantly different ($p<0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 17—The lightness color value (L*) of ground beef patties irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day	Day 7	Day 1	Day 7
0	45.85[a] ± 0.50	46.91[b] ± 0.38	53.99[c] ± 0.11	54.89[c] ± 0.28	43.63[a] ± 0.38	46.33[b] ± 0.34	51.29[c] ± 0.28	53.90[d] ± 0.43	48.13[a] ± 0.25	49.13[a] ± 0.38	55.83[c] ± 0.32	56.79[c] ± 0.44
1.0	46.43[a] ± 0.37	47.21[a] ± 0.28	53.75[c] ± 0.20	54.52[c] ± 0.44	44.73[a] ± 0.69	45.92[a] ± 0.46	49.85[c] ± 0.18	52.91[d] ± 0.72	48.69[a] ± 0.17	50.95[b] ± 0.21	55.05[c] ± 0.36	58.57[d] ± 0.44
1.5	45.56[a] ± 0.13	46.53[a] ± 0.33	53.85[c] ± 0.43	54.22[c] ± 0.42	45.34[a] ± 0.67	45.96[a] ± 035	50.46[c] ± 0.61	52.92[d] ± 0.18	49.06[a] ± 0.19	50.98[b] ± 0.35	54.73[c] ± 0.41	56.83[e] ± 0.25

[1]Mean values within same row (dose) and same column (day) in each replication with different superscripts are significantly different ($p < 0.05$).
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 18—The red-green color value (a*) of ground beef patties irradiated in vacuum and high CO_2 MAP packages

Dose [3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	23.52^{a} ± 0.24	23.36^{a} ± 0.24	33.46^{b} ± 0.39	36.49^{b} ± 0.17	23.62^{a} ± 0.24	23.57^{b} ± 0.31	31.33^{c} ± 0.30	35.48^{d} ± 0.40	23.19^{a} ± 0.14	23.02^{a} ± 0.13	32.23^{b} ± 0.42	35.36^{c} ± 0.40
1.0	23.01^{a} ± 0.19	22.65^{a} ± 0.19	33.56^{b} ± 0.28	34.52^{c} ± 0.28	23.16^{a} ± 0.22	22.53^{a} ± 0.21	31.60^{c} ± 0.29	35.27^{d} ± 0.57	22.31^{a} ± 0.08	22.18^{a} ± 0.10	33.43^{b} ± 0.56	32.99^{b} ± 0.50
1.5	22.79^{a} ± 0.09	22.80^{a} ± 0.26	32.90^{b} ± 0.09	34.23^{b} ± 0.19	22.38^{a} ± 0.17	22.11^{a} ± 0.21	31.01^{c} ± 0.15	33.56^{e} ± 0.53	22.02^{a} ± 0.13	21.57^{a} ± 0.21	32.54^{b} ± 0.26	32.50^{b} ± 0.24

[1]Mean values within same row (dose) and same column (day) in each replication with different superscripts are significantly different ($p < 0.05$).
[2] Each means ± standard error of means
[3]The target irradiation

Table 19--The blue-yellow color value (b*) of ground beef patties irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	Vacuum				MAP			
	Day 1		Day 7		Day 1		Day 7	
	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]
0	14.41[a]	0.06	14.45[a]	0.07	20.03[b]	0.13	21.90[b]	0.18
1.0	14.30[a]	0.08	14.11[a]	0.13	20.17[b]	0.11	21.15[b]	0.27
1.5	14.04[a]	0.08	14.01[a]	0.13	19.81[b]	0.11	20.26[b]	0.17

[1]Mean values within same row (dose) and column (day) with different superscripts are significantly different ($p < 0.05$).
[2] Standard error of means
[3]The target irradiation dose

Table 20--Purge (grams) of ground beef patties irradiated in vacuum and high CO_2 MAP packages

Dose [3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	$2.68^{a,b}$ ± 0.31	3.58^{a} ± 0.54	$1.44^{a,b}$ ± 0.01	$1.57^{a,b}$ ± 0.06	$3.32^{a,b,c}$ ± 0.68	$4.93^{a,b,c}$ ± 0.50	1.71^{b} ± 0.01	$2.86^{a,b,c}$ ± 0.08	4.07^{a-f} ± 0.39	6.06^{a} ± 0.78	$1.84^{b,c,e}$ ± 0.15	$1.81^{b,c,e}$ ± 0.03
1.0	3.46^{a} ± 1.06	$2.94^{a,b}$ ± 0.24	$1.48^{a,b}$ ± 0.02	$1.80^{a,b}$ ± 0.24	$3.84^{a,b,c}$ ± 0.10	$4.30^{a,b,c}$ ± 0.31	1.91^{b} ± 0.20	$5.47^{a,c}$ ± 1.59	3.77^{a-f} ± 0.18	5.29^{a} ± 0.83	$2.42^{b,c,e,d}$ ± 0.14	$2.15^{d,e}$ ± 0.25
1.5	$2.78^{a,b}$ ± 0.31	$3.31^{a,b}$ ± 0.10	1.28^{b} ± 0.08	$1.94^{a,b}$ ± 0.18	$3.60^{a,b,c}$ ± 0.72	$4.19^{a,b,c}$ ± 0.26	$2.70^{a,b,c}$ ± 0.66	$3.10^{a,b,c}$ ± 0.03	5.17^{f} ± 0.89	$4.58^{d,f}$ ± 0.02	$2.30^{b,c,e,d}$ ± 0.04	$1.80^{b,c,e}$ ± 0.05

[1]Mean values within same row (dose) and same column (day) in each replication with different superscripts are significantly different ($p < 0.05$).
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 21--The pH of ground beef patties irradiated in vacuum and high CO_2 MAP packages

Dose [3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	5.73^{a} ± 0.00	5.75^{a,b,d,f} ± 0.01	5.66^{c} ± 0.01	5.74^{a,b} ± 0.02	5.79^{a} ± 0.02	5.45^{b} ± 0.01	5.70^{a,c} ± 0.00	5.55^{b,c} ± 0.01	5.66^{a} ± 0.11	5.82^{a,b} ± 0.00	5.80^{a,b} ± 0.00	5.92^{b} ± 0.03
1.0	5.75^{a,b} ± 0.00	5.77^{a,b,f} ± 0.00	5.70^{c,d} ± 0.00	5.75^{a,b} ± 0.01	5.80^{a} ± 0.03	5.73^{a} ± 0.00	5.71^{a} ± 0.02	5.78^{a} ± 0.05	5.74^{a,b} ± 0.09	5.87^{a,b} ± 0.00	5.80^{a,b} ± 0.05	5.89^{a,b} ± 0.00
1.5	5.74^{a,b} ± 0.00	5.78^{b} ± 0.00	5.72^{b,d,e} ± 0.01	5.77^{b,f} ± 0.00	5.81^{a} ± 0.02	5.82^{a} ± 0.02	5.68^{a,c} ± 0.02	5.77^{a} ± 0.04	5.78^{a,b} ± 0.00	5.88^{a,b} ± 0.00	5.76^{a,b} ± 0.01	5.81^{a,b} ± 0.01

[1]Mean values within same row (dose) and same column (day) in each replication with different superscripts are significantly different ($p < 0.05$).
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 22--Analysis of variance: for TBA values of irradiated ground beef patties packaged in vacuum and high CO_2 MAP

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	42.717	852.870	0.001	0.998
Storage	1	0.518	5.595	0.142	0.737
Packaging	1	0.512	23.260	0.040	0.921
Dose	2	0.256	4.437	0.097	0.689
Rep	2	0.050	0457	0.685	0.307
Storage * Packaging	1	0.051	4.761	0.161	0.704
Storage * Dose	2	0.037	1.056	0.428	0.346
Packaging * Dose	2	0.015	0.725	0.539	0.266
Storage * Packaging * Dose	2	0.012	4.183	0.105	0.677
Storage * Rep	2	0.093	2.178	0.208	0.464
Packaging * Rep	2	0.022	0.785	0.506	0.243
Storage * Packaging * Rep	2	0.011	3.826	0.118	0.657
Dose * Rep	4	0.058	1.111	0.425	0.399
Storage * Dose * Rep	4	0.035	2.328	0.016	0.925
Packaging * Dose * Rep	4	0.020	7.162	0.041	0.877
Storage * Packaging * Dose * Rep	4	0.003	0.483	0.748	0.018

Table 23--The TBA values of ground beef patties irradiated in vacuum and high CO_2 MAP packages

Dose [3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	0.50^{a} ± 0.02	0.43^{a} ± 0.00	0.48^{a} ± 0.03	$0.58^{a,b}$ ± 0.00	$0.35^{a,e}$ ± 0.00	$0.37^{c,e}$ ± 0.02	$0.54^{b,d,f}$ ± 0.04	$0.63^{f,g}$ ± 0.02	$0.40^{a,e}$ ± 0.02	$0.43^{b,e}$ ± 0.01	0.34^{e} ± 0.00	$0.54^{a,b,c}$ ± 0.07
1.0	0.50^{a} ± 0.02	0.55^{a} ± 0.00	0.55^{a} ± 0.00	0.80^{b} ± 0.11	$0.38^{c,e}$ ± 0.01	$0.57^{b,d,f}$ ± 0.06	$0.60^{b,f}$ ± 0.01	0.76^{g} ± 0.01	$0.50^{d,e}$ ± 0.03	$0.68^{c,d}$ ± 0.07	$0.60^{b,c}$ ± 0.02	0.83^{d} ± 0.03
1.5	0.46^{a} ± 0.03	0.45^{a} ± 0.04	$0.58^{a,b}$ ± 0.06	$0.63^{a,b}$ ± 0.06	$0.46^{c,d,e,h}$ ± 0.02	$0.46^{b,e}$ ± 0.03	$0.51^{b,c,f}$ ± 0.05	$0.52^{b,f,h}$ ± 0.01	$0.44^{b,e}$ ± 0.04	0.80^{d} ± 0.02	$0.53^{c,e}$ ± 0.03	0.86^{d} ± 0.06

[1]Mean values within same row (dose) and same column (day) in each replication with different superscripts are significantly different ($p < 0.05$).
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 24—LS means[1,2] ± standard errors for sensory attributes of irradiated ground beef patties in vacuum or high CO_2 MAP packages

Packaging	Red color[4]	Irradiated off-Aroma[4]	Sour-like aroma[4]	Raw ground beef aroma[4]
Vacuum	$2.1^{a} \pm 0.5$	3.5 ± 0.6	1.5 ± 04	$4.4^{a} \pm 0.6$
MAP[3]	$10.9^{b} \pm 0.5$	4.3 ± 0.6	1.6 ± 0.4	$3.4^{b} \pm 0.6$

[1] Data for irradiation treatments were pooled since no interaction between packaging and irradiation effects was observed
[2] Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3] Modified atmosphere packaging.
[4] Line scale, numerical value of 15; none=0; intense =15.

Table 25—LS means[1,2] ± standard errors for sensory attributes of raw ground beef irradiated at different doses

Dose (kGy)	Red color[3]	Irradiated off-aroma[3]	Sour-like aroma[3]	Raw ground beef aroma[3]
0	6.9 ± 0.5	2.4^{a} ± 0.6	1.1 ± 0.5	4.7^{a} ± 0.7
1.0	6.5 ± 0.5	5.0^{b} ± 0.6	1.8 ± 0.5	3.2^{b} ± 0.7
1.5	6.2 ± 0.5	4.4^{b} ± 0.6	1.7 ± 0.5	$3.7^{a,b}$ ± 0.7

[1]Data for packaging treatments were pooled since interaction between and irradiation effects was observed
[2]Means in a column followed by a different superscriptions are significantly different ($p<0.05$).
[3]Line scale, numerical value of 15; none=0; intrense=15.

Table 26—LS means[1,2] ± standard errors for sensory attributes of cooked ground beef patties irradiated in vacuum or high CO_2 MAP packages prior to cooking

Packaging	Sour-like aroma[4]	Ground beef aroma[4]	Juiciness[5]	Sourness[4]
Vacuum	$0.3^{a} \pm 0.2$	$7.5^{a} \pm 0.6$	8.7 ± 0.5	1.0 ± 0.3
MAP[3]	$0.7^{b} \pm 0.2$	$5.9^{b} \pm 0.6$	8.1 ± 0.5	1.0 ± 0.3

[1]Data for irradiation treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2]Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3]Modified atmosphere packaging
[4]Line scale, numerical value of 15; none=0; intense =15
[5]Line scale, numerical value of 15; not juicy=0; very juicy=15.

Table 27—LS means[1,2] ± standard errors for sensory attributes of cooked ground beef patties irradiated at different doses prior to cooking

Dose (kGy)	Sour-like aroma[3]	Ground beef aroma[3]	Juiciness[4]	Sourness[3]
0	0.3 ± 0.2	7.9[a] ± 0.7	7.9 ± 0.6	0.8 ± 0.3
1.0	0.6 ± 0.2	6.1[b] ± 0.7	8.5 ± 0.6	1.0 ± 0.3
1.5	0.7 ± 0.2	6.1[b] ± 0.7	8.9 ± 0.6	.1 ± 0.3

[1] Data for packaging treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2] Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3]Line scale, numerical value of 15; none=0; intense =15.
[4]Line scale, numerical value of 15; not juicy=0; very juicy=15.

Table 28—LS means[1,2] ± standard errors for sensory attributes of cooked ground beef patties in vacuum and high CO_2 MAP packages and at different irradiation doses prior to cooking

Treatment	Irradiated off-aroma[4]	Irradiated off-flavor [4]	Ground beef flavor [4]
Control-Vacuum	$1.9^{c} \pm 0.7$	$1.8^{c} \pm 0.8$	$7.5^{a,b} \pm 0.7$
1.0 kGy-Vacuum	$2.6^{b,c} \pm 0.7$	$2.2^{b,c} \pm 0.8$	$6.8^{a,b} \pm 0.7$
1.5 kGy-Vacuum	$3.1^{a,b,c} \pm 0.7$	$3.4^{b,c} \pm 0.8$	$6.5^{a,b} \pm 0.7$
Control-MAP[3]	$1.4^{c} \pm 0.7$	$1.2^{c} \pm 0.8$	$8.0^{a} \pm 0.7$
1.0 kGy-MAP	$5.4^{a} \pm 0.7$	$6.6^{a} \pm 0.8$	$4.0^{c} \pm 0.7$
1.5 kGy-MAP	$5.1^{a} \pm 0.7$	$4.8^{a,b} \pm 0.8$	$5.3^{b,c} \pm 0.7$

[1] An interaction was noted between the packaging and irradiation treatments. Individual treatment means are, therefore reported.
[2] Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3] Modified atmosphere packaging.
[4] Line scale, numerical value of 15; none=0; intense =15.

CHAPTER 3−CONTROL OF *LISTERIA MONOCYTOGENES* ON FRANKFURTERS AND PRE-COOKED PORK CHOPS BY IRRADIATION COMBINED WITH MODIFIED ATMOSPHERE PACKAGING

A paper to be revised, condensed and submitted to Meat Science

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Abstract

The efficacy of controlling *Listeria monocytogenes* on frankfurters and pre-cooked pork chops with irradiation and modified atmosphere packaging (MAP) containing a high concentration of carbon dioxide (100% CO_2) was investigated in this study. Frankfurters and pre-cooked pork chops were inoculated with a five strain cocktail of *L. monocytogenes* and packaged in vacuum or high CO_2 MAP. Irradiation was applied to the product at 0, 0.5, 1.0 or 1.5 kGy. There was no significant packaging effect for the radiation sensitivity of *L. monocytogenes* in this study. Radiation D_{10}-values for *L. monocytogenes* were 0.66 ± 0.03 kGy and 0.70 ± 0.05 kGy on frankfurters, and 0.60 ±

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0.02 kGy and 0.57 ± 0.02 kGy on pre-cooked pork chops, each in vacuum and high CO_2 MAP, respectively. High CO_2 MAP in this study was more effective for controlling of the growth of survivors during refrigerated storage (for at least 12 weeks) than vacuum packaging (up to 7-9 weeks). Lipid oxidation was very limited in both of these two irradiated RTE meats in either vacuum or MAP. The pH of the products was not affected by either irradiation or packaging technique. There was no irradiation-induced redness in pre-cooked pork chops packaged in high CO_2 MAP as was observed for chops in vacuum packaging. Irradiation and high CO_2 MAP has potential to control *L. monocytogenes* in RTE meats. However, high concentration of CO_2 in MAP produced gas pockets in frankfurters during cooking, which adversely affected the texture of the product. Furthermore, a sour-like aroma and sour taste were observed for samples in CO_2 MAP which along with irradiated off-aroma observed in these products present challenges to overcome in order to apply this technology.

Keywords: *Listeria monocytogenes*; Ready-to-eat meat; Irradiation; Modified atmosphere packaging

1. Introduction

Post-processing contamination with *Listeria monocytogenes* on ready-to-eat meat (RTE meat) products is one of the biggest current concerns for public health by the meat industry. According to statistical analysis from the Centers for Disease Control and Prevention (CDC), among 2500 cases of listeriosis each year in the U.S., 90% of the cases are caused by consumption of ready-to-eat (RTE) food products contaminated with *L. monocytogenes* (Mead et al., 1999). The fatality rate of listeriosis is about 20%, primarily targeting immunocompromised persons, elderly persons, pregnant women and

their fetuses (Gellin & Broome, 1989; Ryser & Marth, 1991). Deli meat and non-reheated frankfurters have been catergorized as high risk for causing listeriosis (FDA, USDA-FSIS & CDC, 2001). Hence it is written in the federal law that "zero tolerance" applies to *L. monocytogenes* in RTE meat products in the U.S. (USDA-FSIS, 2007). For this reason, there has been tremendous economic loss by the processed meat industry due to the recalled products that were possibly contaminated with *L. monocytogenes* (Murphy et al., 2006). After the multistate outbreak of listeriosis caused by contaminated turkey deli meat in 2002, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) issued a final rule in 2003 for the processed meat industry to develop three scientifically validated alternative programs for the control of *L. monocytogenes* in RTE meat and poultry products (USDA-FSIS, 2003; Gottlieb et al., 2006). The final rule requires that *L. monocytogenes* be considered as hazard and is included in Hazard Analysis and Critical Control Point (HACCP) plans of processed meat establishments. At the same time, the USDA-FSIS increased the inspection and sampling in establishments producing RTE meat to provide incentives for the industry to increase the testing for this organism and incorporate other preventive measures to control or eliminate this food-borne pathogen (USDA-FSIS, 2005).

L. monocytogenes is an environmental survivor. This facultative gram-positive psychrotroph is resistant to many harsh environments including dry, cold, heat, salty and vacuum conditions (Zhu et al., 2005a). The biofilm that this organism forms on food processing surfaces, equipment and other production environmental surfaces creates a great challenge for sanitation procedures to completely eradicate this pathogen from food processing facilities (Beresford, Andrew & Shama, 2001; Somers & Wong, 2004; Thevenot, Dernburg & Vernozy-Rozand, 2006). Cross contamination between processing

environments and RTE meat products can occur during post-processing handling and packaging procedures (Lin et al., 2006; Vorst, Todd & Ryser, 2006). The current common practices in the processed meat industry to control and/or prevent post-processing contamination are thermal lethality treatments, such as pre- or post-packaging pasteurization, or nonthermal treatments, such as formulating or dipping products with sodium or potassium lactate and sodium diacetate as bacteriostatic agents in the processed meats (Seman et al., 2002; Stekelenburg, 2003; Muriana et al, 2004). Current research has focused on combining lethality methods and listeriostatic methods to control this pathogen during post-processing packaging, and to inhibit the growth of survivors during storage, for instance, combining organic acids or other antimicrobial treatments with post-packaging pasteurization (Luchansky, Cocoma & Call, 2006; Murphy et al., 2006). When applying intervention strategies to control food-borne pathogens in meat products, the effect on the product quality has been a major concern, for example, post-packaging pasteurization can increase purge, increase fat and moisture loss, and change the color of products (Murphy et al., 2001; Selby et al., 2006). Therefore, the ultimate goal of food pathogen intervention strategies should be to ensure both safety and quality of products (Juneja, 2004).

Food irradiation is a well-established non-thermal lethal technology to inhibit food-borne pathogens in not only fresh, but also processed meat products (Olson 1995; Fu, Sebranek & Murano, 1995; Sommers et al., 2004). However, irradiation, especially at medium doses (1.5 to 2.0 kGy), may also cause quality changes, including off-odor, changed meat color and lipid oxidation in processed meat products, and those changes are directly related to irradiation dose (Fan, Sommers & Sokorai, 2004; Houser et al., 2005a). Furthermore, after irradiation, *L. monocytogenes* survivors may grow during refrigerated

storage (Foong, Gonzalez & Dickson, 2004). Therefore, many studies have been conducted on the combination of additional hurdles with irradiation to minimize negative quality side effects by reducing irradiation dose, and to improve control of the growth of pathogen survivors during the storage. One of the approaches that have been studied is the combination of irradiation with modified atmosphere packaging (Lee et al., 1996).

Modified atmosphere packaging (MAP) with either low (20-30%) or high (60-100%) carbon dioxide content has been used for inhibiting spoilage bacteria in meat and to extent the shelf life of the product (Rao & Sachindra, 2002). Many reports have shown that MAP with high CO_2 is more effective than low CO_2 for control of spoilage bacteria in fresh meats, and meat shelf life was longer in MAP with high CO_2 (Buys et al., 1994; Holley et al., 1994; Tewari et al., 1999; Patsias et al., 2006). It was also observed that high CO_2 MAP inhibited the growth of some foodborne pathogens, including *Escherichia coli O157:H7*, *Salmonella*, *L. monocytogenes* and *Campylobacter jejuni,* in meat products (Marshall et al., 1991; Farber, Cai & Ross, 1996; Nissen et al., 2000; Dykes et al., 2001, Olarte et al., 2002; Michaelsen, Sebranek & Dickson, 2006). There are also some reports which have shown that, compared with vacuum packaging, no effect of high CO_2 MAP on the growth of *L. monocytogenes* in RTE food products during the storage (Claire, et al., 2004; Tovunac et al., 2005). Chiasson, Borsa & Lacroix (2005) observed that when ground beef was packaged in MAP with 30% CO_2, 60% O_2 and 10% N_2, the radiation sensitivity of *E. coli O157:H7* and *Salmonella* Typhimurium were increased. Thayer & Boyd (1999, 2000) reported that high concentration of CO_2 in MAP inhibited the recovery and the growth of *L. monocytogenes* in turkey meat during 28 days of post-irradiation storage. Low oxygen MAP with CO_2 also has the advantage of excluding oxygen from packages and preventing lipid oxidation often caused by irradiation. For

example, Grant and Patterson (1991) reported that the combination of 1.75 kGy irradiation with MAP containing 25% CO_2 (balanced with N_2) produced the best combination of microbiological and sensory quality in pork chops. Therefore, combining irradiation with low oxygen and high CO_2 MAP might be an effective hurdle intervention strategy to improve control of the growth of *L. monocytogenes* during storage, and to maintain the quality of irradiated RTE meats as well. Few studies have been done on the radiation sensitivity of *L. monocytogenes* on RTE meat products packaged in high CO_2 MAP, or on the survival and recovery of this foodborne pathogen in high CO_2 MAP at refrigeration temperature, or with temperature abuse following irradiation treatments.

The objective of this study was to test the hypothesis that irradiation combined with high CO_2 MAP (100% CO_2) is more effective than irradiation with vacuum packaging for reducing *L. monocytogenes* on frankfurters (cured meat product) and cooked pork chops (uncured meat product), and for inhibiting the growth of survivors at 2-4 ºC or with temperature abuse. Quality and sensory evaluations were also included to assess the quality implications of the combined treatments.

2. Materials and methods

2.1. Experimental design

This study was conducted as two experiments. Experiment 1 was designed to determine and compare the irradiation sensitivity (D_{10}-value) of *L. monocytogenes* in frankfurters or cooked pork chops packaged with either vacuum or high CO_2 MAP packaging, and to evaluate survivor growth during storage at 2-4 ºC and at 25 ºC (temperature abuse). Experiment 2 was designed to determine and compare quality and

sensory attributes of irradiated frankfurters and cooked pork chops packaged with vacuum or with high CO_2 MAP packaging.

A random block design was used for both experiments. A 2 × 4 factorial design was used for treatments in experiment 1 to determine radiation sensitivity (D_{10}-values) of *L. monocytogenes* on frankfurters and cooked pork loins, and to assess the survivor growth status during storage. Vacuum and MAP comprised two levels of packaging while irradiation doses of 0 kGy (control), 1.0 kGy, 1.5 kGy and 2.0 kGy comprised four levels of irradiation treatment. A 2 × 3 factorial design was used for sensory evaluation in experiment 2. Vacuum and MAP comprised two levels of packaging with irradiation doses of 0 kGy (control), 1.0 kGy and 2.0 kGy as three levels of irradiation treatment. A 2 × 3 × 2 factorial design was used for color, purge, pH and rancidity evaluations. The two packaging treatments (vacuum and MAP), three irradiation doses (0 kGy, 1.0 kGy and 2.0 kGy) and two storage times (the first day and the 7^{th} day of storage for cooked pork chops; and the first day and 28^{th} day of storage for frankfurters) after irradiation were used for these assessments. There were three samples included for each treatment in experiment 1 and two samples for each treatment in experiment 2. Both experiments were repeated three times.

2.2. Preparation of bacterial cultures

Five strains of *L. monocytogenes* were used to make a cocktail to represent different serotypes of this pathogen. These cultures were supplied by the Iowa State University Food Safety Research Laboratory (ISU-FSRL) and included H7764 (serotype 1/2a, food product isolate), serotype 1/2a FSIS (food product isolate), H7762 (serotype 4b, food product isolate), H7969 (serotype 4b, food product isolate), and Scott A

(serotype 4b, clinical isolate). Stocks were separately transferred to 10 ml Tryptic Soy Broth with 0.6 % yeast extract (TSBYE) (Difco, Becton Dickinson, Sparks, MD, U.S.A.) and incubated at 35 ºC for 24 hours. One milliliter of the cultures was then transferred into 99 ml TSBYE and incubated at 35 ºC for 24 hours. The concentration of the bacteria reached about 7 log cfu /ml.

2.3. Preparation of meat samples

Frankfurters were manufactured in the USDA-inspected Meat Laboratory at Iowa State University (ISU). The raw meat used to make frankfurters contained beef trim (90% lean) and pork trim (50% lean). The total fat content of the raw meat was determined with an Anyl-Ray Fat Analyzer (Kartridg Pak Co. Davenport, IA, U.S.A.), and averaged 29%. Other ingredients used to make frankfurters included water, sodium chloride, dextrose, mustard flour, coriander, ground black pepper, ground nutmeg, garlic powder, sodium tripolyphosphate, sodium nitrite and sodium erythorbate. Enhanced pork loins (injected with water, sodium chloride, phosphate, and potassium lactate) were purchased from a local manufacturer. Pork loins were pre-cooked and sliced into 1.5 cm thick chops in the ISU Meat Laboratory. Single slices of cooked pork chops (about 100 gram /chop) for both experiment 1 and 2 were placed into high barrier pouches (Curlon Grade 861, 3cc O_2 / 645 cm2 / 24 h at 23 ºC and 0% RH; Cryovac Division, W.R. Grace Co., Duncan, SC, U.S.A.). Frankfurters (6 links per package) were packaged in the same pouches. Frankfurters or pre-cooked pork chops for inoculation were immediately transferred to the Pathogen Laboratory in the ISU-FSRL.

2.4. Inoculation and packaging

For the inoculation of frankfurters in experiment 1, the five *L. monocytogenes* cultures were combined (500 ml) into a sterilized Prex pan as the inoculum. Frankfurters were immersed in the inoculum for 2 min, and then aseptically transferred to high barrier pouches (6 links /pouch) which were the same as those used for pork chops. The concentration of the bacteria attached to each frankfurter was approximately 4-5 log /cm^2 (the average surface area of a frankfurter was 84 cm^2) according to preliminary tests. For the inoculation of cooked pork chops, the inoculum was prepared by combining 20 ml of each culture in a 100 ml sterilized dilution bottle. The cocktail contained approximately equal numbers of each strain with a total concentration of bacteria of 7 log / ml. One milliliter of inoculum was placed on the pork chop in each pouch with a sterilized pipette. The packages were manually massaged for 1-2 min to distribute the inoculum evenly. The concentration of the bacteria on each pork chop was approximately 5 log /gram. Pouches were immediately vacuum- or MAP-packaged with a Multivac (model A 300/52) packaging machine (Multivac Inc, Wolfertschwenden, Germany) in the FSRL. Cylinders with the desired gas mixture (100% CO_2) for MAP packaging were purchased from Linweld Co. (Linweld Co., Lincoln, NE, U.S.A.). The MAP packaging was done by programming the Multivac packaging machine with vacuum (10-13 mbars) for the pouches first, and then flushing the gas mixture into the pouches (pressure of 680-700 bars) with simultaneous sealing. The volume ratio of gas to the frankfurters and cooked pork chop in a single MAP package was about 2:1 and 4:1, respectively. After inoculation and packaging, samples were stored at 2-4 ºC for 12 hours before irradiation.

Uninoculated frankfurters and pre-cooked pork chops for experiment 2 were packaged in the ISU Meat Laboratory using pouches (see experiment 1) and a Multivac

(model C500) packaging machine (Multivac Inc, Wolfertschwenden, Germany). The packaging machine was a different machine than that used for inoculated samples, however, the vacuum and gas packaging procedures were the same. Frankfurters were packaged as six links per package, and pork chops as one per package.

2.5. Irradiation

The inoculated, packaged samples for experiment 1 were irradiated at the Iowa State University Linear Accelerator Facility (ISU-LAF). The irradiation was generated by a Circe-III linear electron accelerator at an energy level of 10 MeV and 10 kW (MeV Industries S.A., Jouy-Josas, Cedex, France). The target irradiation doses were 1.0, 1.5 and 2.0 kGy. Alanine pellet dosimeters (5 mm × 5 mm) (Broker Analytische Messtechnik, Rheinstetten, Germany) were placed on the top and bottom surface of sample pouches to measure the actual absorbed energy (dose). Immediately after irradiation, the absorbed doses were measured by electron paramagnetic resonance on a Broker EMS 104 EPR Analyzer. The average surface dose, overall average dose and average maximum doses absorbed by frankfurters or pork chops in vacuum and MAP are listed in Table 1.

Following irradiation, the samples were stored at 2-4 ºC in the ISU-FSRL Pathogen Laboratory.

Uninoculated samples for experiment 2 were irradiated at the same facility (ISU-LAF) as the inoculated samples, but at a different time. The target doses were 1.0 kGy and 2.0 kGy. The average surface dose, overall average dose and average maximum doses absorbed by frankfurters or pork chops in vacuum and MAP are listed in Table 2.

Following irradiation, the samples were stored at 2-4 ºC prior to quality evaluation. The samples for sensory evaluation were transferred to ISU Sensory Evaluation Center immediately following irradiation.

2.6. Determination of D_{10}-value

Plating was conducted immediately after irradiation treatment (within 1 hour). The samples were massaged manually from outside of the pouches. For irradiated frankfurters, one of the six frankfurters in a package was aseptically transferred from the package into a sterile plastic stomacher bag (Whirl-Pack filter bag B01318, Nasco, Fort Atkinson, WI, U.S.A.) with 84 ml of peptone water (Difco, Becton Dickinson, Sparks, MD, U.S.A.), homogenized with a Stomacher blender (Seward Stomacher Blender, Model 4000, Tekmar Co., Cincinnati, OH, U.S.A.) for 60 seconds at slow speed and surface plated onto plates of Oxford formulation *Listeria* Selective Agar, including selective supplement (MOX agar) (Oxoid, Basing-stock, U.K.) after series dilution. The plates were incubated at 35ºC for 48 hours before counting. For irradiated, cooked pork chops, the entire pork chop was aseptically cut into small pieces and transferred into a sterile plastic stomacher bag (same as the stomacher bags used for frankfurters) with peptone water to make the initial 50% dilution. After homogenization with the Stomacher blender for 60 seconds at high speed, the series dilutions were surface plated onto MOX agar plates. The plates were incubated at 35ºC for 48 hours before counting. Irradiation D_{10}-value is defined as the amount of radiation energy (dose) needed to reduce 90% (cfu) of the microorganisms in irradiated food products (Thayer et al., 1986). The D_{10}-value was determined by calculating the negative reciprocal of the slope of the regression line

of the plot of log number of survivors (log cfu / gram or cm^2) versus irradiation dose (kGy) (Foong, Gonzalez & Dickson, 2004).

2.7. Enumeration of survivors during storage

Recovery of the pathogen in irradiated frankfurters and cooked pork chops was measured after 24 and 48 hours of storage at 2-4 ºC, and at 1 week intervals for 12 weeks of storage to determine the growth pattern of the survivors. For the temperature abuse test, samples were first held for 14 days at 2-4 ºC, and then followed by room temperature (25 ºC) for 48 hours prior to enumeration. The plating method was the same as or determination of D_{10}-values.

2.8. Color Measurement

CIE color values (L*, a* b*) of the product surfaces were measured with a Hunter Lab LabScan (Model LS 1500, Hunter Associated Labratories Inc., Reston, VA, U.S.A.). CIE standard illuminant A (incandescent or tungsten lamplight), 10 degree observer and a 1.75-inch port insert were used. The temperature of the light source is 2,856 ºK. The exterior and interior color (inside of a longitudinal section) of the frankfurters was measured after opening the packages. The color of pre-cooked pork chops was measured on the packaged samples through the packaging material. Three readings were randomly collected from different locations on each sample. Measurements were conducted on day 1 and day 7 after irradiation for cooked pork chops, and on day 1 and day 28 after irradiation for frankfurters.

2.9. Package Purge

Purge was measured by weighing the empty pouches before packaging. After irradiation, the weight of the packaged sample with packaging material was recorded prior to opening the package. Then, the sample was removed, dried with paper towels and weighed. The quantity of purge was determined by subtracting the weight of the packaging film and the weight of the sample from the weight of the packaged sample before it was opened.

2.10. Sample pH

The pH of the samples was measured with a FC 200B pH electrode (Hanna Corporation, Hanna USA, www.hannainst.com) at 25 ºC immediately following the purge measurement by direct insertion of the electrode into the samples.

2.11. Oxidative Rancidity

Lipid oxidation status of pre-cooked pork chops was assessed using the 2-thiobarbituric acid (TBA) distillation procedure (Tarladgis et al.,1960). For frankfurters, a modified TBA method for cured meats (Zipser & Watts, 1962) was used to determine the oxidative status. Absorbance of the chromophore produced by the reaction between 2-thiobarbituric acid and malonaldehyde (one of the lipid oxidation products) at 532 nm was automatically converted to mg of malonaldehyde per kg of sample by a computerized Beckman Du 640 spectrophotometer (Beckman Coulter, Canada). Duplicate TBA values per sample were recorded.

2.12. Sensory evaluation

Sensory evaluations of frankfurters and pre-cooked pork chops were conducted using a ten-member sensory panel of faculty, staff, and students at Iowa State University. All panelists were volunteers and the project was approved by the Iowa State University Human Subjects Review Committee. Panelists were trained to evaluate the sensory attributes of the products in two one-hour training sessions. Each panelist evaluated six samples per session and three sessions each were conducted for frankfurters and for pre-cooked pork chops. A computerized sensory scoring system (COMPUSENSE five, v 4.4, Compusense, Inc. Guelph, Ontario, Canada N1H3N4) was used to collect sensory evaluation data.

A. Frankfurters: Sensory evaluation of both unheated and heated frankfurters was conducted. The unheated frankfurters were evaluated for color and aroma and the heated frankfurters were evaluated for aroma, appearance, texture, and flavor.

For unheated frankfurters, individual packages of chilled (4ºC) frankfurters (6 links /package) with random three-digit codes were simultaneously presented to panelists on trays in a randomized order. Panelists were instructed to cut open the package as close to the sample as possible, wait 3-5 seconds, and smell the sample. The samples were presented simultaneously and panelists evaluated the samples in the randomized order presented on the computer screen. Testing was conducted in partitioned booths and under red fluorescent lights. Samples were evaluated for irradiated off-aroma, sour-like off-aroma, and unheated frankfurter aroma using a 15-unit numerical line scale with descriptive anchors (left anchor-none, right anchor-intense) at each end of the line. Following each of the aroma sessions, the panelists evaluated the color of frankfurters from each treatment. The frankfurters on white foam trays in their original packages

were labeled with random three digit codes, and were placed on a white paper background in randomized order. The samples were evaluated under white florescent lighting positioned to provide 70 foot-candles at the counter surface. A six-point category scale was used to evaluate color differences between the frankfurter samples and the control (vacuum packaged, non-irradiated). The category scales were identified as no difference, very slight difference, slight difference, moderate difference, large difference, and very large difference.

For evaluating heated frankfurters, the frankfurters were placed in a covered saucepan containing boiling water for 7 minutes. Each panelist received one-half of a frankfurter, with ends cut off, in a foam container labeled with a random three-digit code. Samples were served immediately after heating and cutting. Cooking/serving orders were randomized. Testing was conducted in partitioned booths, under red fluorescent lights. Panelists evaluated the sensory attributes in the following order: irradiated off-aroma, frankfurter aroma, denseness, firmness, irradiated off-flavor, sourness, and frankfurter flavor. A 15-unit numerical line scale was used for scoring. For aroma and flavor, the left anchor was labeled as low intensity (none) and the right anchor as high intensity (intense). For denseness (by appearance, not by mouth-feel), the left anchor was labeled as not dense and the right anchor as very dense. For firmness, the anchor labels were not firm and very firm.

B. Pre-cooked pork chops: The sensory evaluation of pre-cooked pork chops also included unheated and heated samples. Unheated chops were evaluated for color and aroma, and the heated chops were evaluated for aroma, texture, and flavor.

For unheated chops, individually cooled chops (4ºC) in the original packages were labeled with random three-digit codes, and were simultaneously presented to the

panelists on trays in a randomized order. Panelists were instructed to cut open the package as close to the sample as possible, wait 3-5 seconds, and smell the sample. A reference sample (vacuum/0 kGy) was served to each of the panelists prior to serving the test samples. Testing was conducted in partitioned booths and under red fluorescent lights. Samples were evaluated for irradiated off-aroma, sour-like aroma, and unheated cooked pork aroma. A 15-unit numerical line scale was used with descriptive anchors (left anchor-none, right anchor-intense). Following each of the aroma sessions, the panelists evaluated the color of a single pork chop from each treatment. The refrigerated chops in the original packages on white ceramic plates with random three digit codes were placed on a white paper background in a randomized order. The chops were evaluated under white florescent lighting positioned to provide 70 foot-candles at the counter surface. Panelists evaluated the intensity of pink color and brown color with a 15-unit numerical line scale with the left anchor labeled as none and the right anchor labeled as intense.

For the heated product, the cooked pork chops on a ceramic plate were re-heated in a microwave oven for 55 seconds. Then, the chops were inverted and heated for another 55 seconds. The internal temperature of chops when microwave heat was completed was 55 ± 3 ºC, monitored with a thermocouple (Chromega/Alomega) attached to an Omega digital thermometer (Model DSS-650, Omega Engineering). Two chops per treatment were prepared, cut into 15 mm cubes, and the cubes mixed. Panelists received two randomly selected cubes in covered 4-ounce foam containers labeled with random three-digit codes. Samples were served immediately after cutting and mixing. Cooking/serving orders were randomized. Testing was conducted in partitioned booths and under red fluorescent lights. A 15-unit numerical line scale with descriptors of low

intensity (none) at the left and high intensity (intense) at the right was used for scoring irradiated off-aroma, sour-like aroma, pork aroma, irradiated off-flavor, sourness, and pork flavor. Firmness (left anchor-not firm, right anchor-very firm and juiciness (left anchor-not juicy, right anchor-very juicy) was also evaluated.

2.13. Statistical Analysis

A general linear model (SPSS 14.0 Window Grad Pack) was used to evaluate the effects of irradiation dose, packaging types and storage time. When there were significant effects or interactions ($p<0.05$) between experimental factors, linear contrast test, independent sample T-test or post-hoc tests of differences with Tukey adjustment were used to analyze the significance of main and simple main effects, or simple-simple main effects.

A mixed linear model was fit with PROC MIXED (SAS Inst., Inc., Cary, N.C., U.S.A, version 9.1) to determine the effects of irradiation dose and packaging technique on the sensory attributes. A random subject term was fitted to incorporate subject-to-subject variability. When a fixed effect was significant ($p<0.05$), post-hoc tests of differences were calculated and then adjusted with the Tukey procedure.

3. Results and discussion

3.1. Radiation D_{10}-values

Table 3 shows that there was no significant packaging effect on the radiation sensitivity of *L. monocytogenes* on frankfurters (p value: 0.619) or cooked pork chops (p value: 0.137). There was no interaction between treatments or between replications

(ANOVA not shown). D_{10}-values for this bacterium on frankfurters were 0.66 ± 0.03 kGy with vacuum packaging, and 0.70 ± 0.05 kGy with high CO_2 MAP. On pre-cooked pork chops, the D_{10}-values were 0.59 ± 0.02 kGy with vacuum packaging, and 0.57 ± 0.02 kGy with high CO_2 MAP. These results are consistent with the observation of Thayer and Boyd (1999), who reported that CO_2 in MAP had no effect on radiation sensitivity of *L. monocytogens* inoculated in turkey meat. These authors also reported that *L. monocytogenes* was more sensitive to irradiation in aerobic packaging than in vacuum or MAP with or without oxygen. In the present study, the D_{10}-values of this organism on frankfurters (cured meat) were greater than on pre-cooked chops (uncured meat). However, Fu, Sebranek & Murano (1995) reported that *L. monocytogenes* on cured hams was more sensitive than on uncured pre-cooked pork chops (0.38 kGy for ham v.s. 0.30 kGy for chops), although these D_{-10} values were smaller than the D_{10}-values obtained in our experiments. These authors also reported that there was no effect of the injected brine on the radiation sensitivity of this pathogen. It was not clear whether the lower D_{10}-value observed on pre-cooked pork chops in the present study was affected by potassium lactate (antimicrobial) in the chops. Foong, Gonzalez & Dickson (2004) investigated the radiation sensitivity of *L. monocytogenes* on six different types of RTE meats, including frankfurters, ham, roast beef, bologna, and smoked turkey with or without lactate. The D_{10}-values were reported to range from 0.42-0.44 kGy; and these authors did not observe any effect of lactate (in smoked turkey) or curing agent (in frankfurters, ham and bologna) on the radiation sensitivity of *L. monocytogenes*. Zhu et al. (2005b) also reported that there was little effect on radiation sensitivity of *L. monocytogenes* when several combinations of antimicrobials (sodium lactate, sodium diacetate, potassium benzoate) were formulated into turkey hams. However, Sommers et al. (2003) reported that sodium

diacetate and potassium lactate in beef bologna increased radiation sensitivity of *L. monocytogenes* in this product. These authors reported a D_{10}-value of 0.56 kGy for bologna without these antimicrobials, and 0.46 kGy for bologna with 0.15% sodium diacetate and 2% potassium lactate. Sommers & Fan (2003) also reported that sodium diacetate increased the radiation sensitivity of *L. monocytogenes* on fine-emulsion sausages. These reports suggested that antimicrobials in emulsified meats, such as bologna and frankfurters, may have more effect on radiation sensitivity of *L. monocytogenes* than when added to unemulsified RTE meats, such as hams, roast beef or pork chops. These observations also show that D_{10}-values of *L. monocytogenes* may vary when the meat matrix is different. Sommers & Thayer (2000) observed that there was a significant effect of meat type and other protein ingredients, such as soy protein, in a frankfurter formulation on radiation sensitivity of *L. monocytogenes*. These authors reported D_{10}-values ranging from 0.49-0.71 kGy for this pathogen when surface-inoculated on frankfurters with different meats in the formulation, such as beef franks, mixed species franks, poultry franks, etc. *L. monocytogenes* on mixed species frankfurters was most irradiation resistant (D_{10} value: 0.71 ± 0.09). The frankfurters used in the present study were made from mixture of pork and beef; the D_{10}-value of this pathogen was consistent with that reported by these authors. Further more, Gursel & Gurakan (1997) observed that *L. monocytogenes* was more resistant to gamma irradiation in fresh minced beef (D_{10} value: 0.699 kGy) than in minced fresh chicken breast meat (D_{10} value: 0.599 kGy); and Thayer et al. (1998) observed that the D_{10}-value of *L. monocytogenes* on cooked turkey breast meat was significantly higher than on raw turkey breast (0.69 ± 0.03 kGy vs 0.56 ± 0.03 kGy).

In summary, there was no packaging (vacuum or high CO_2 MAP) effect on radiation sensitivity of *L. monocytogenes* on frankfurters or cooked pork chops in the present study. However, when radiation D_{10}-values of *L. monocytogenes* on processed meat products are reported, other factors should be also taken into consideration. Different meat types, additives such as soy protein, antimicrobials, curing agents (sodium nitrite), salt content (sodium chloride), fat content, pH, water activity of products and product temperature were also reported to affect irradiation D_{10}-values of bacteria (Buchanan, Stahl & Whiting, 1989; Huhtanen, Jenkins & Thayer, 1989; Thayer & Boyd, 1995; Dickson, 2001; Luchansky et al., 2002). Further more, different bacterial strains used for inoculating the test samples, the sampling size, different histories of inoculum and different media (selective or unselective) used for recovery of bacteria also affect the estimation of radiation D_{10}-values of *L. monocytogenes* in RTE meat products (Patterson, 1989; Tarte, Murano and Olson, 1996; Ngutter & Donnelly, 2003; Foong, Gonzalez & Dickson, 2004; Mendonca et al, 2004).

3.2. Recovery of L. monocytogenes

Counts of *L. monocytogenes* on frankfurters or cooked pork chops following irradiation (day 1) were compared with the counts after 24 hours (day 2) and 48 hours (day 3) at 2-4 ºC to determine the recovery of the survivors at refrigeration temperature. According to the results from analysis of variance (ANOVA not shown), there was no packaging effect (p-value: 0.466), or time (day) effect (p-value: 0.838) on counts of *L. monocytogenes* in this study; however, there was an interaction between dose, time and replication (p-value: 0.017). Therefore, the means (log cfu /cm^2) of each treatment in each replication are presented in tables 4, 5 and 6. Table 4 shows growth of this

bacterium in both control (vacuum and MAP) packages after 24 and 48 hour stored at 2-4 °C in replicate 1. Table 5 shows a decrease in the bacterial numbers only in vacuum packages irradiated at 2.0 kGy after 24 and 48 hours storage in replicate 2. There was no change of the cell counts observed in replicate 3 (Table 6). Because these results were not consistent in three replications, we considered the changes in cell numbers to be caused by enumeration variation rather than experimental treatments, and concluded that there were no significant changes in the counts of *L. monocytogenes* as a result of packaging environments after 24 or 48 hours at 2-4 °C.

Table 7 shows the ANOVA results for the recovery of *L. monocytogenes* on irradiated pre-cooked pork chops. There was a significant time (day) effect (p-value: 0.006), but no packaging effect (p-value: 0.067) on the population of *L. monocytogenes* on chops. There were also four way interactions between packaging, time, dose and replications (p-value: 0.018). Interestingly, t-test showed no significant packaging or time effects on counts of *L. monocytogenes* on irradiated or non-irradiated pre-cooked pork chops when the data from three individual replications was separately analyzed (data not shown). Hence, there were no significant changes in the counts of *L. monocytogenes* on irradiated or non-irradiated pre-cooked pork chops after 24 or 48 hours at 2-4 °C, neither in vacuum, nor in high CO_2 MAP packaging. The recovery data from this study were used to compare these results with the survival curve of the broth-based anaerobic growth model for *L. monocytogenes* in the USDA Pathogen Modeling Program (Version 7.0). Parameters of 4.0 ºC, pH 5.8 (average pH for frankfurters) or pH 6.4 (average pH for enhanced cooked pork chops) and sodium chloride concentrations of 2.5% (for frankfurters) or 0.5% (the lowest concentration in this model) for pork chops, and 150 ppm (the highest value in the model) of sodium nitrite (for frankfurters) were used for

predicting the pathogen growth in irradiated frankfurters or cooked pork chops. According to the model, there should have been some growth under the conditions included for cooked pork chops (lag phase: 2.12 days), but not under the conditions included for frankfurters. Walker, Archer & Banks (1990) reported that the minimum growth of *L. monocytogenes* in chicken broth or UHT milk was -0.1 to -0.4 °C, and that the organism grew at 5 °C after 1-3 days of incubation. However, Glass & Doyle (1989) suggested that the recovery and survival of *L. monocytogenes* on processed meats stored at refrigeration temperature (4.4 °C) was product-dependent, and that even the same product from different manufacturers may result in large variation when assessing the fate of this microorganism. In the model developed by Farber, Cai & Ross (1996), the growth of *L. monocytogenes* in MAP packaging was dependent on the product pH, storage temperature, and the concentration of CO_2 in MAP. The organism did not grow in brain heart infusion broth for up to 30 day at 4 °C when the pH was 5.5, and when CO_2 was greater than 50 %. In the present study, although the concentration of CO_2 in MAP was 100%, the effect of CO_2 was not obvious during the short period of recovery (24 or 48 hours) at refrigeration temperature, because *L. monocytogenes* on pre-cooked pork chops did not grow in either vacuum or CO_2 MAP. This result was consistent with the observation in the Thayer and Boyd study (1999). These authors reported that *L. monocytogenes* on irradiated turkey meat packaged in either vacuum or high CO_2 MAP did not increase significantly until after 5 days of storage at 7 °C. Thayer et al. (1998) also reported that the population of *L. monocytogenes* on irradiated cooked turkey breast or cooked ground turkey did not increase within 24 and 48 hours of storage at 4 °C. Some studies have suggested that irradiation can not only reduce the bacteria numbers, but also delay the recovery afterward. Foong, Gonzalez & Dickson (2004) reported that after

irradiation with 2.0 kGy, *L. monocytogenes* did not grow on RTE meats (frankfurters, ham, roast beef, bologna, and smoked turkey with or without lactate) until the fifth week of storage at 4 °C.

3.3. The growth of L. monocytogenes during refrigerated storage

Table 8 shows the ANOVA results for *L. monocytogenes* on frankfurters (irradiation or non-irradiated) during refrigerated storage (4 °C) for 12 weeks. There was a significant effect (p-value: 0.000) of storage (week), but no significant effect of packaging type (p-value: 0.116). However, there were interactions between dose, week, packaging and replications (0.001). Therefore, the results (log cfu /cm^2) from each replication are presented in tables 9 to 14.

Replicate 1: The results (tables 9 &10) show that *L. monocytogenes* on control-vacuum (0 kGy-vacuum) packaged frankfurters increased significantly at the 9^{th} week of storage. There was an increase of the population in some of 1.0 kGy-vacuum packages (not all packages) at the 10^{th} and 11^{th} week of storage. In 1.5 kGy-vacuum packages the cell counts increased at the 9^{th} week of storage. There was no significant change in the cell count for the 2.0 kGy-vacuum packages through 12 weeks of storage. The population of *Listeria monocytogenes* in all high CO_2 MAP packages of this replication did not change, irrespective of irradiation dose.

Replicate 2: The results (tables 11& 12) show that in control-vacuum packages, the *L. monocytogenes* population increased significantly by the 7^{th} week of storage, and then remained relatively steady until the 12^{th} week. In 1.0 kGy-vacuum packages, the numbers of this bacterium began to exceed initial counts at the 7^{th} week and became significant by the 10^{th} week of storage. In 1.5 kGy-vacuum packages, the bacteria

numbers rose gradually following the 8th week and became significantly greater by the 11th week. In some of 2.0 kGy-vacuum packages (not all packages), the cell numbers appeared to increase by 1-2 log at the 11th and 12th week of storage, although the growth was not statistically significant due to the large variation of cell counts. As in replicate 1, the population of *L. monocytogenes* did not change in any high CO_2 MAP packages through 12 weeks of storage.

Replicate 3: The results (tables 13&14) show that in control-vacuum packages, the population of *L. monocytogenes* again increased significantly at the 7th week of storage, and then remained steady through the rest of the storage time. In 1.0 kGy and 1.5 kGy-vacuum packages, the cell numbers of this pathogen increased significantly by the 8th week of storage. The bacterium also grew in 2.0 kGy-vacuum packages following the 8th week of storage. As in the previous two replications, the population of *L. monocytogenes* did not change in any packages of high CO_2 MAP through 12 weeks of storage.

Table 15 shows the ANOVA results for the population of *L. monocytogenes* on pre-cooked pork chops (irradiation or non-irradiated) during refrigerated storage (4 °C) for 12 weeks. There were significant effects of packaging (p-value: 0.017) and storage (week) (p-value: 0.040). However, there were also interactions between dose, packaging, storage and replications (p-value: 0.004). Therefore, the results (log cfu /gram) from each replication are presented in tables 16 to 21.

Replicate 1: The results (table 16) show that *L. monocytogenes* in control-vacuum packages gradually increase after the 4th week of storage and became significantly greater at the 10th week. In the 1.0 kGy-vacuum packages, the cell numbers increased significantly at the 11th week of storage. In 1.5 kGy-vacuum packages, the

bacterium increased gradually after the 2nd week with growth becoming significant at the 11th week. In the 2.0 kGy-vacuum packages, multiple statistical comparisons did not show any significant changes in the cell counts due to the large variation, but a small increase is suggested by the data at end of storage (3-6 log cfu /gram). In high CO_2 MAP packages (table 17), *L. monocytogenes* increased by about 1 log in control-MAP packages at the end of storage. There was no significant growth of this microorganism in irradiated MAP packages through 12 weeks of storage.

Replicate 2: The results (table 18) show that *L. monocytogenes* in control-vacuum packages increased significantly at the 9th week. In the 1.0 kGy-vacuum packages, the bacterium number became significantly greater at the 7th week, and in the 1.5 kGy and 2.0 kGy-vacuum packages, large variation again resulted in no significant change in the population of *L. monocytogenes*. For the high CO_2 MAP packaging (table 19), in control samples, and in the 1.0 kGy and 1.5 kGy-MAP packages, the population of *L. monocytogenes* did not change significantly through 12 weeks, while the cell numbers in the 2.0 kGy-MAP packages were reduced after the 7th week of storage.

Replicate 3: The results (table 20) show that there was no statistically significant change of the population in control-vacuum treatment during storage due to large variation in the data. The growth of this microorganism in the 1.0 kGy and 1.5 kGy-vacuum packages became significant at or after the 7th week, respectively. For high CO_2 MAP packaging (table 21), there was no significant change of the population of *L. monocytogenes* in the control, 1.0 kGy or 1.5 kGy-MAP packages, while in the 2.0 kGy-MAP packages, the counts of this bacteria deceased gradually. In some of the packages, there were no viable cells detected after the 3rd week of the storage.

The overall results of this study showed that *L. monocytogenes* can proliferate in vacuum packaged irradiated or non-irradiated frankfurters or pre-cooked pork chops during 12 weeks of refrigerated storage (4 °C). The lag phase for this microorganism on vacuum packaged non-irradiated or irradiated frankfurters at 1.0 kGy or 1.5 kGy was 7-10 weeks. The growth of this pathogen in the 2.0 kGy-vacuum packages was not consistent, with growth observed in some of 2.0 kGy-vacuum packages in all three replications with lag phases ranging from the 4^{th} week to the 11^{th} week of refrigerated storage. High CO_2 MAP packaging in the present study was more effective than vacuum packaging during storage, and inhibited the growth of this foodborne pathogen at least 12 weeks at refrigeration temperature. These results are similar Thayer & Boyd (1999) who reported that high CO_2 MAP packaging prevented the multiplication of *L. monocytogenes* on irradiated (2.0 kGy) turkey meat for 28 days at 7 °C. Michaelsen, Sebranek & Dickson (2006) also observed that high CO_2 MAP controlled the growth of *L. monocytogenes* on hams for 28 days of storage at 4 °C. However, in the present study, no synergetic effect of irradiation and high CO_2 in MAP on inhibition of *L. monocytogenes* on either frankfurters or pre-cooked pork chops could be determined, because the high CO_2 MAP alone (control-MAP) controlled the growth of this microorganism for at least 12 weeks during storage.

The growth model for *L. monocytogenes* in the USDA Pathogen Modeling Program (PMA, version 7.0) shows that the lag phase of this pathogen in broth-based anaerobic conditions is predicted to be 2.12 days at 4 °C with 0.5 % sodium chloride at pH 6.4 for cooked pork chops. The lag phase duration is predicted to be 12.99 days at 4 °C in the presence of sodium nitrite (150 ppm, the highest value in the model), 2.5 % sodium chloride and pH 5.8 for frankfurters. It should be noted that potassium lactate,

which was injected in the pork chops in the present study, is not included in the PMA. This antimicrobial agent is likely to extend the lag phase of *L. monocytogenes* on vacuum or MAP packaged pork chops during storage. Many studies have showed this effect of lactate and diacetate on control of *L. monocytogenes* on RTE meats. Glass et al. (2002) reported that the multiplication of *L. monocytogenes* on cooked bratwurst (non-smoked & uncured) was inhibited by 2 % sodium lactate for 28 days at 3 °C. Sommers & Fan (2003) studied the combination of irradiation with addition of sodium diacetate for beef bologna to control *L. monocytogenes.* These authors reported that the growth of this pathogen in 1.5 kGy-vacuum packaged bologna with 0.25% sodium diacetate was delayed for 2 weeks at 9 °C. Zhu et al. (2005b) also observed that 1.0 kGy irradiation combined with 2 % sodium lactate and 0.1% sodium diacetate extended the lag phase of *L. monocytogenes* on turkey ham for 6 weeks at 4 °C. Furthermore, in the present study, the lag phase of this pathogen was significantly longer in high CO_2 MAP than in vacuum packages; this result suggested that CO_2 in MAP and potassium lactate might be synergistic for control of *L. monocytogenes* on pork chops used in the present study. Devlieghere et al (2001) suggested in the predictive model that the concentration of sodium lactate and the amount of CO_2 absorbed in cooked meat products had a synergistic effect on control of the growth of *L. monocytogenes* in MAP packaged RTE meat. However, Michaelsen, Sebranek and Dickson (2006) reported that potassium lactate and sodium diacetate formulated in hams were more effective for control of *L. monocytogenes* in vacuum packaging than in high CO_2 MAP at 4 °C.

Many studies have demonstrated that sodium nitrite contained in cured RTE meat, including frankfurters, has inhibitory effects on *L. monocytogenes* (Whiting & Masana, 1994). Buchanan, Stahl & Whiting (1989) observed that five process and storage factors

(temperature, pH, atmosphere, sodium chloride and sodium nitrite) interacted to affect the growth of *L. monocytogenes* in tryptose phosphate broth. These authors reported that sodium nitrite had significant bacteriostatic activity at pH 6.0, temperature below 5 °C, 4.5% or more sodium chloride and an anaerobic atmosphere; however, sodium nitrite did not contribute to this function when pH was above 7. After investigating vacuum packaged salami, sliced corned-beef, ham and lunch meat from 15 stores over a period of one year, Grau & Venderlinde (1992) reported that the amount of residual nitrite contained in RTE meat was crucial for the control of *L. monocytogenes* during refrigeration, transportation and retail sale. These authors observed that the product water activity and pH also interplayed with residual nitrite to inhibit the growth of this microorganism. For instance, during 6 weeks of refrigerated storage, on RTE meat products with a_w above 0.97 and pH above 6, the growth of *L. monocytogenes* was slowest when the residual nitrite was 170 ppm, and fastest when residual nitrite was 5 ppm. Birzele, Djordjevic & Kramer (2005) also reported that high concentration of residual nitrite (above 40 ppm) inhibited the growth of *L. monocytogenes* on fresh spreadable ham and onion sausage for at least 15 days at 8 °C. Therefore, the residual nitrite in vacuum or MAP packaged frankfurters (average pH was 5.8) in the present study might be one of the factors that extended the lag phase of *L. monocytogenes* during the refrigerated storage, although the concentration of residual nitrite in frankfurters was not determined. Houser et al. (2005b) reported that the concentration of residual nitrite in cured ham (156 ppm sodium nitrite added) decreased from 15.3 ppm (in non-irradiated ham) to 13.7 ppm when the product was irradiated with 4.5 kGy. These authors also reported that residual nitrite continued to be depleted with the time of storage in the vacuum packaged ham. In the present study, the lag phase of *L. monocytogenes* on

frankfurters was longer in high CO_2 MAP than in vacuum packaging; therefore, information on how much residual nitrite was depleted with the storage time and how the residual nitrite interacted with CO_2 in MAP to control *L. monocytogenes* would provide valuable information for future predictive models.

Many studies have been done on treatments for control of *L. monocytogenes* on vacuum packaged irradiated RTE meats with or without sodium nitrite. Fu, Sebranek & Murano (1995) reported that the growth of *L. monocytogenes* on irradiated vacuum packaged cooked pork chops (irradiated at 0.76 kGy) or ham (irradiated at 0.9 kGy) was delayed for 7 days at 7 °C; however, the growth rate of this pathogen was much lower on ham than on cooked pork chops. Thayer et al. (1998) reported that *L. monocytogenes* grew on irradiated cooked turkey breast meat (3 kGy) within 21 days at 2 or 7 °C. In the study by Patterson, Damoglou & Buick (1993) reported that this organism was able to grow on poultry meat irradiated with 2.5 kGy after 18 days of storage at 6 °C. However, in the study of Foong, Gonzalez & Dickson (2004), the growth of *L. monocytogenes* on several kinds of irradiated (2.0 kGy) RTE meats (including cured and non-cured products) was inhibited for 5 weeks at 4 °C, and for 1 week at 10 ° C. While nitrite appears to be important, these reports do not fully explain the effect of sodium nitrite on the growth of this bacterium because the irradiation dose, the storage temperature and products in each report were different.

The background microflora on frankfurters and cooked pork chops was not assessed in the present study during the storage period. However, after 8-10 weeks of storage at 4 °C, the growth of spoilage microorganisms was observed on the surface of cooked pork chops as yellow colonies, a slimy surface, and strong off-odor. Thayer & Boyd (2000) reported that the growth rate of *L. monocytogenes* at 7 °C was the same on

MAP packaged ground turkey meat with original background microflora as on MAP packaged irradiated ground turkey meat with fewer background bacteria. According to Radin, Niebuhr & Dickson (2006), the presence of spoilage microflora on frankfurters can affect the growth of *L. monocytogenes* at 10 °C, irrespective of the number of spoilage microflora. Therefore, the background bacteria may also be one of the factors that affected the lag phase duration of *L. monocytogenes* on RTE meat during refrigerated storage.

Although many reports have shown the effectiveness of high CO_2 MAP for control of *L. monocytogenes* on different kinds of products, the interplay between product ingredients and the environment of the product has not been adequately studied. Therefore, further study is needed to investigate the interactive effects of irradiation, packaging (including vacuum and high CO_2 MAP), antimicrobials (including concentrations of residual nitrite), and background microflora on the growth of *L. monocytogenes* in RTE meat products at refrigeration temperature.

3.4. The growth of L. monocytogenes during temperature abuse

Table 22 (ANOVA) shows that there was neither a packaging effect (p-value: 0.085) nor a temperature effect (p-value: 0.195) on the growth of *L. monocytogenes* on non-irradiated or irradiated vacuum or MAP packaged frankfurters held at room temperature (25 °C) for 48 hours following 2 weeks of storage at 4 °C. There were interactions between irradiation dose, temperature, packaging type (p-value: 0.001); between irradiation dose, temperature, and replication (p-value: 0.001); between irradiation dose, packaging, and replication (p-value: 0.016), and between temperature, packaging and replication (p-value: 0.029). However, t-test indicated that there was no

significant change in the population of *L. monocytogenes* on frankfurters in either vacuum or MAP packages after 48 hours temperature abuse (data not shown)

Table 23 shows the ANOVA results for the growth of *L. monocytogenes* on non-irradiated or irradiated vacuum or MAP packaged cooked pork chops held at room temperature (25 °C) for 48 hours following 2 weeks refrigerated storage. There was no packaging (p-value: 0.096) or temperature effect (p-value: 0.238); however, there was interaction between experimental factors. Further, the results from individual replications (tables 24-26) show some growth in vacuum packaged samples. For instance, in replicate 2, there were significant increases in the population of *L. monocytogenes* in control-vacuum, 1.0 kGy and 1.5 kGy-vacuum packages during temperature abuse.

The growth model for *L. monocytogenes* in the USDA Pathogen Modeling Program (PMA, version 7.0) predicts that within 24 hours *L. monocytogenes* will grow to 7-8 log cfu /ml under conditions typical for frankfurters (pH 5.8, 3.0% sodium chloride, 150 ppm sodium nitrite, and 25 °C) if initial cell numbers are 5 log cfu /ml; or will multiply to 5-6 log cfu /ml if initial cell numbers are 3 log cfu /ml. The reports from many studies were also in accordance with the prediction of PMA. For instance, Fu, Sebranek & Murano (1995) observed that *L. monocytogenes* reached about 8 log cfu /g on irradiated ham (irradiated at 0.76 kGy) after temperature abuse at 25 °C for 48 hours following 7 days of storage at 7 °C. These authors also reported that 2.0 kGy of irradiation reduced the initial 6 log cfu /g on ham to undetectable levels; however, after 2 days of temperature abuse at 25 °C for 48 hour, some injured cells were able to recover and could be detected. However, in the present study, the cell numbers of this bacterium declined slightly in 2.0 kGy-vacuum or 2.0 kGy-MAP packages during the temperature abuse. Chen et al. (2004) reported that *L. monocytogenes* grew on irradiated (2.3 kGy)

frankfurters with pediocin in the formulation after 2 days exposure to room temperature (25 °C). Michaelsen, Sebranek & Dickson (2006) also observed that *L. monocytogenes* in vacuum packaged ham (without addition of antimicrobials) grew after 5 days of storage at 10 °C. Therefore, in the present study, it is not clear why *L. monocytogenes* did not grow at 25 °C on non-irradiated or irradiated frankfurters packaged in either vacuum or MAP packaging.

The USDA PMP predicts that for conditions similar to pre-cooked pork chops (pH 6.4, 0.5% sodium chloride, 0 sodium nitrite, and 25 °C), *L. monocytogenes* can reach 8-9 log cfu /ml after 24 hours if initial cell numbers are 3 log cfu /ml (in anaerobic broth base). In the present study, the growth of this organism on pork chops was inhibited in all of the high CO_2 MAP packages during temperature abuse. The population of this organism in the control-vacuum, 1.0 kGy and 1.5 kGy-vacuum packages reached 4-5 log cfu /gram in one of three replications, but no growth in the 2.0 kGy-vacuum packages was observed. *L. monocytogenes* was not able to grow in most of 2.0 kGy-vacuum packages. These results suggest that potassium lactate in the pre-cooked pork chops may have contributed to control of *L. monocytogenes* when the product was exposed to temperature abuse, although this antimicrobial seemed less effective at room temperature than at refrigeration temperature. The relationships of temperature and the efficacy of sodium lactate for control of the growth of *Lactobacillus sake* in cooked meat products packaged in MAP were described in the model developed by Devlieghere et al. (2000). This model predicted that sodium lactate would be more effective for control of this lactic acid bacterium in MAP at low refrigerated temperature. In the present study, both products were stored at 4 °C for two weeks before being placed at room temperature, therefore, *L. monocytogenes* might already be injured by CO_2, potassium lactate or nitrite

(Jay, Loessner & Golden, 2005); hence, this pathogen might not be able to recover within 48 hours during the temperature abuse. Furthermore, in the present study, there may be an irradiation dose effect or an interaction between irradiation and potassium lactate on the growth of this bacterium on irradiated pork chops, because the growth was inhibited in the 2.0 kGy vacuum packages. High CO_2 MAP in the present study was more effective than vacuum packaging for the control of growth of *L. monocytogenes* on pre-cooked pork chops during temperature abuse for at least two days. An interactive effect of potassium lactate and high CO_2 in MAP might also have occurred in this study, although Michaelsen, Sebranek & Dickson (2006) observed that using high CO_2 in MAP along with potassium lactate and sodium diacetate in ham did not differ from CO_2 alone or animicrobials alone for control of *L. monocytogenes* at 10 °C. Nevertheless, according to predictions with the model developed by Devlieghere et al. (2001), temperatures above 7 °C are in the *L. monocytogenes* "risk areas" for cooked meat products containing 3% sodium lactate and packaged in CO_2 MAP packaging. This occurs because the absorption of CO_2 into the product will decrease rapidly with increasing temperature and the synergistic effect of CO_2 and sodium lactate will be also reduced at the same time.

Many studies have shown that carbon dioxide or organic acid salts (including potassium lactate, sodium lactate, sodium diacetate, etc.) have greater antimicrobial function at lower temperature. For instance, Eklund & Jarmund (1983) reported that the growth inhibition of *E. coli* and *Salmonella* by CO_2 increased when temperature deceased from 20 °C to 6°C and 2 °C. Lu et al. (2005) observed that the effect of organic acid salts on control of *L. monocytogenes* on frankfurters was greater at 1.1 °C than at 4.4 °C, 10°C or 12.8 °C. However, Jakobsen & Bertelsen (2004) observed that in addition to dissolving in the water phase, CO_2 can also dissolve in the fat of meat products. The

model developed by these authors predicted that when storage temperature is above 2 °C, the solubility of CO_2 is positively related to the fat content of meat products. Therefore, if meat products in CO_2 MAP are stored at temperature above 2 °C, the total amount of CO_2 absorbed in meat products should include the quantity in both water phase and fat phase. However, it is not clear if CO_2 dissolved in fat has the same bacteriostatic function as in aqueous phase. Further, storage at 4 °C for two weeks before exposure to room temperature (simulating the actual cases of mishandling RTE meats), may cause the behavior of bacteria in CO_2 MAP with antimicrobials to be different from what is simulated in many predictive models where meat products are directly placed at different temperatures immediately after the production or packaging. Therefore, the recovery or growth of *L. monocytogenes* on frankfurters or pre-cooked pork chops during the temperature abuse may also be different from what is predicted by the models.

Overall, it is clear that the effects of product packaging are affected by many other factors, including meat type, different processing procedures (including irradiation), product formulation (including antimicrobials), product pH, storage temperature, water activity of the product and more. A more complex model is needed to include all these factors and to better predict the growth of this microorganism during refrigerated transportation, storage or in the event of temperature abuse.

3.5. Color values

Both external and internal color values (CIE L*, a* and b*) of irradiated and non-irradiated frankfurters were assessed. Table 27 shows that the exterior L* value (lightness value) of frankfurters packaged in high CO_2 MAP was less (darker) immediately following irradiation than to frankfurters packaged in vacuum (p-value: 0.000). However,

after 28 days of storage, the lightness value for the frankfurters was almost the same in all packages. There was no significant main effect of any experimental factors according to ANOVA , however, there were interactions between irradiation dose, storage time, packaging type and replication (p-value: 0.025; other ANOVA results not shown). Therefore, the exterior a* values (red-green color value) of frankfurters in each replication is presented in table 28. In a few cases, irradiated frankfurters (in both vacuum and MAP packages) became less red after 28 days of storage, but this result was not consistent in the three replications. Most of the frankfurters retained redness throughout the storage period and were largely unaffected by irradiation or packaging treatment. The exterior b* value (yellow-blue color value) of the frankfurters was not affected by irradiation, packaging or storage (data not shown).

There was no significant effect of irradiation dose, packaging or storage on interior lightness (L* value) of frankfurters (data not shown). For the interior a * value for the frankfurters, there was no significant main effect of experimental factors, however, results between replications were different (p-value: 0.036; other ANOVA results not shown). Therefore, the results for each replication are presented in table 29. The interior redness of frankfurters in two of three replications was not affected by any of the experimental factors. The interior yellow-blue color (b*) was the same in all packages, irrespective of irradiation dose, packaging or storage (data not shown).

Results from three replications showed that irradiation, packaging and storage did not have significant effect on the overall color value of frankfurters.

For the pre-cooked pork chops, there was no significant main effect on the lightness (L*). The results from each replication are presented in table 30, because of the interaction between all experimental factors (p-value: 0.000). In two of the three

replications, the lightness (L*) of the chops did not differ. For the redness of pre-cooked pork chops, there were significant main effects of irradiation (p-value: 0.037) and packaging type (p-value: 0.047). There was also interaction between irradiation dose and packaging type (p-value: 0.000). The interactions between experimental factors and replications were also significant (p-value: 0.22), therefore, the results from each replication are presented in table 31. The results show that irradiation, irrespective of the dose, increased the redness at day 1 of pre-cooked pork chops in vacuum, but not in high CO_2 MAP packages. However, in two of the replications, the red color of irradiated chops in vacuum packages decreased to the same level as the control-vacuum or MAP packages after 7 days of storage. Further, the pork chops in vacuum were also more yellow after storage (p-value: 0.002 & 0.017) in two of the replications (table 32); however, this did not occur in the MAP packages.

3.6. Package Purge and pH

There was a significant packaging effect on the pH of frankfurters (p-value: 0.011; other ANOVA results not shown). Since there were also interactions between irradiation dose, packaging type, storage and replication (p-value: 0.037), data from each replication is presented in table 33. Interestingly, in one of the three replications, the pH of frankfurters was higher in the high CO_2 MAP packages than in vacuum packages prior to storage. In all three replications, the pH of frankfurters in both vacuum and MAP packages was significantly lower after 28 days of storage (p-value: 0.000-0.042) than at the beginning of storage. However, there was no significant main effect of irradiation, packaging or storage on the pH of pre-cooked pork chops (table 34).

There was a significant packaging effect on the package purge of frankfurters (p-value: 0.007; other ANOVA results not shown). Table 35 shows that the amount of package purge for frankfurters was significantly greater in vacuum packages than in MAP packages. Table 36 shows that the amount of package purge of cooked pork chops was slightly higher in vacuum packages than in MAP packages, although ANOVA (not shown) did not show any significant effects due to the relatively large variation in the data.

3.7. Oxidation rancidity

There was no significant effect of experimental factors on the TBA value of frankfurters. However, there were interactions between irradiation dose, packaging type, storage and replication (p-value: 0.000; other ANOVA results not shown). Therefore, the results from each replication are presented in table 37. While the TBA of frankfurters in some MAP packages was significantly higher within a replication than in vacuum packages, the TBA values of all treatments were well below 1.0, which is often suggested as a threshold of oxidative rancidity in meat products. Irradiation did not affect the TBA value. There was no significant main effect of experimental factors on the TBA value of pre-cooked pork chops. The results from each replication for the chops are presented in table 38 due to in interactions between irradiation dose, packaging type, storage and replication (p-value: 0.000). While a few treatment combinations resulted in greater TBA values, the TBA values of cooked pork chops in all treatments were below 1.0.

3.8. Sensory evaluation

Tables 39 and 40 show that irradiation did not affect the sensory attributes of unheated frankfurters; however, frankfurters from high CO_2 MAP packages had more intensive sour-like aroma and less intense frankfurter aroma in comparison to frankfurters from vacuum packages. Tables 41 and 42 show that the sensory attributes of heated frankfurters were affected by irradiation and packaging techniques. All irradiated frankfurters had more intense frankfurter aroma and frankfurter flavor and were denser than non-irradiated samples. Irradiated frankfurters from MAP packages had less intensive irradiated off-aroma than from vacuum packages, and also had more intensive sour like-aroma, less intensive frankfurter flavor and were less dense and less firm.

Tables 43, 44 and 45 show that the sensory attributes of unheated pre-cooked pork chops were affected by irradiation and packaging techniques. Irradiated chops had more intensive irradiated off-aroma and less intensive cooked pork chop aroma. Cooked chops from MAP packages also had more intensive irradiated off-aroma, more sour-like aroma and less pork chop aroma. Irradiated chops from vacuum packages had more intensive pink color which was irradiation dose-dependent. Table 59 shows that the heated pork chops from MAP packages were less firm and juicier, but had stronger sour taste than those from vacuum packages.

Although many studies have reported that the color of cured RTE meats can be affected by irradiation along with irradiation-induced lipid oxidation (Fu, Sebranek & Murano, 1995; Sommer & Fan, 2003; Houser et al., 2005a, 2005b), the overall color or lipid oxidation status of frankfurters in the present study was not changed by irradiation. However, the greater redness induced by irradiation in vacuum packaged pre-cooked

pork chops was consistent with many reports. For example, Fan, Sommers & Sokorai (2004) observed that irradiation (1.5 and 3.0 kGy) induced redness in turkey bologna without nitrite in the formulation. Zhu et al. (2004) reported that irradiation (1.0-1.2 kGy) increased the a* value of turkey breast roll. Byun et al. (1999) have studied the use of irradiation (5 kGy) in place of nitrite to generate red color in hams. These authors observed that the red color induced by irradiation was similar to the cured color produced with 200 ppm nitrite.

In the present study, the irradiation induced-redness on pre-cooked pork chops in vacuum packages measured as CIE a* was not dose-dependent; however, the intensity of redness assessed by the sensory panel was dose-dependent. Furthermore, the redness of the chops decreased during 7 days of storage. This result was similar to that reported by Nam et al. (2006). These authors reported that irradiation (2.5 kGy) induced redness in pre-cooked restructured pork loin slices, and the redness deceased after 10 days of storage. However, while irradiation will increase redness in pork and poultry (raw or cooked); it also reduces the redness in beef and lamb (Luchsinger et al., 1996; Nanke, Sebranek & Olson, 1998, 1999; Millar, Moss & Stevenson, 2000). Nam & Ahn (2002a, 2002b) and Nam et al. (2006) suggested that irradiation-induced pink color in raw turkey meat, pre-cooked turkey rolls, and pre-cooked restructured pork loins was caused by a carbon monoxide-myoglobin complex formed in the reduced environment induced by irradiation, especially in an anaerobic environment, such as vacuum packaging. The redness of irradiated turkey and pork products was dose-dependent. However, it is not clear where the carbon monoxide originates or which forms of myoglobin (deoxy, oxy, or metmyglobin) may be involved in cooked meat products during irradiation treatment. In our study of high CO_2 MAP containing 0.5 % CO, it was evident that carbon monoxide

does not bind to oxymyoglobin or metmyoglobin, but will only bind with deoxymyoglobin produced by vacuum to form carboxymyoglobin (bright cherry red color). In our experiment, carbon monoxide was not as reactive in the meat system in our experiment as in living human or animal blood and muscle systems, where carbon monoxide can easily replace oxygen as the ligand bound to the sixth position of ferrous heme iron. For meat, it was necessary to remove oxygen from the meat system by vacuum, and then CO was able to bind to the heme iron and produce red color. Therefore, although irradiation could reduce metmyoglobin to myoglobin in fresh pork or poultry products (Nam & Ahn, 2002a), it is not clear why irradiation can also induce redness in turkey or chicken meat even under aerobic conditions. Furthermore, since the high CO_2 MAP treatment was packaged by first removing air with vacuum twice before flushing with 100% CO_2, the concentration of residual oxygen in high CO_2 MAP (tested in preliminary studies, data not shown) was equal to or less than that in vacuum packages. Therefore, the oxidation-reduction potential in high CO_2 MAP should be similar to the vacuum packages at least on the day of irradiation as reported by John and other (2005). However, there was no irradiation-induced redness observed in the pre-cooked pork chops packaged in high CO_2 MAP. Therefore, further study is needed to investigate why irradiation did not induce the red or pink color in cooked pork chops packaged in the modified atmosphere with high carbon dioxide.

In the present study, three factors contributed to limiting lipid oxidation in irradiated frankfurters and cooked pork chops: anaerobic packaging (vacuum or high CO_2 MAP), phosphate and nitrite in the formulation of the products. Ahn et al. (2001) studied the packaging effect on lipid oxidation in cooked turkey, beef and pork. These authors suggested that vacuum packaging of meat after cooking was one of the crucial factors for

control of rancidity in irradiated cooked meat products. Shahidi, Pegg & Shamsuzzaman (1991) observed that polyphosphate had an antioxidant function and facilitated control of lipid oxidation in nitrite-free cured meat irradiated at 5 or 10 kGy. Zhao and Sebranek (1996) observed that dipping pork chops in sodium tripolyphosphate solution improved the lipid stability after irradiation. Byun et al. (1999) and Fan, Sommers & Sokorai (2004) observed that nitrite contributed on antioxidant function to reduce lipid oxidation in irradiated pork or turkey products.

The pH of either frankfurters or pre-cooked pork chops in this study was not significantly different as a result of irradiation or packaging. Although the pH of frankfurters in both vacuum and MAP packaging decreased after 28 days of storage (pH 5.72-5.89), it was still within the normal pH range of meat products (Aberle et al., 2001). However, the sensory panel detected a sour aroma (smell) and a sour taste in frankfurters packaged in MAP despite the fact that little pH difference was found between the packaging treatments. Martinez et al. (2005) reported that the pH of pork sausage packaged in high CO_2 MAP was significantly decreased; however, the sensory properties of the product were not affected. Sorheim, Ofstad & Lea (2004) suggested that a high concentration of CO_2 in MAP decreased the pH of ground beef, increased the purge and cooking loss of the product

The pH of pre-cooked pork chops in both packaging treatments was higher than the pH of frankfurters. The average pH of pork chops was close to 6.5. The pH of enhanced pork loins used for making pre-cooked pork chops is affected by addition of phosphate (Aberle et al., 2001). The greater pH may have limited purge in cooked pork chops, which was very small, and was not affected by irradiation dose or packaging. Pre-cooked pork chops from high CO_2 MAP packages had more intensive sour aroma and

sour taste than those were from vacuum packages, despite the high pH of the product. Holley et al. (1994) suggested that CO_2 in MAP might cause reduction of the surface pH of pork loin slices, although the pH of inside muscle was not changed significantly. If that was also the case for the products (frankfurters or pre-cooked pork chops) in the present study, low surface pH of the product might cause sour aroma or sour taste while the pH of ground, blended samples prepared for analysis did not show any significant overall pH reduction.

Bruce et al. (1996) and Sorheim, Ofstad & Lea (2004) observed that CO_2 was absorbed into beef in high CO_2 MAP packages and then evolved rapidly during cooking to produce large pores in the meat due to gas formation. A similar phenomenon was observed in the present work when frankfurters packaged in high CO_2 MAP were placed at room temperature or were heated. This may explain the sensory panel assessment of frankfurters from high CO_2 MAP as less dense and less firm compared to frankfurters packaged in vacuum.

Irradiation off-aroma in processed meat products has been extensively studied (Ahn et al., 1998, 1999; Du & Ahn, 2002; Lee and Ahn 2003; Houser et al., 2005a). The radiolytic volatiles, such as sulfur and carbonyl compounds, produced by irradiation, are major contributors to the off-odor of irradiated meat products. Many studies have reported that irradiated off-odor was not dose dependent and cannot be reduced by adding antioxidants in the product formulation (Fu, Sebranek & Murano, 1995; Fan, Sommers & Sokorai , 2004; Nam et al., 2006). In the present study, irradiation enhanced frankfurter aroma according to the sensory panel. The irradiated off-odor was less in frankfurters from high CO_2 MAP packages than those from vacuum packages. For irradiated pre-cooked pork chops, irradiation off-aroma was more intense in un-heated chops from high

CO_2 MAP packages than those from vacuum packages. According to comments from the panelists, the sour aroma enhanced the irradiated off-odor in the chops. However, irradiated off-odor or off-flavor was the same in all irradiated samples after heating, irrespective of packaging.

4. Conclusions

The microbiological results of this study indicated that radiation sensitivity of *L. monocytogenes* was not increased by high CO_2 MAP. Irradiation combined with high CO_2 atmosphere packaging was similar to irradiation combined with vacuum packaging for elimination of *L. monocytogenes* on RTE meat products. This result disproves the original hypothesis that the combination of irradiation with high CO_2 MAP will reduce the pathogen from RTE meat more effectively. High CO_2 MAP, however, was more effective than vacuum for controlling the growth of survivors during long term refrigerated storage. The quality characteristics of RTE meat as indicated by pH and TBA values were not affected by packaging technique. The high CO_2 in MAP may not be feasible for packaging frankfurters, because CO_2 produced gas pockets (pores) in frankfurters, so that the frankfurters became less dense and less firm compared to frankfurters packaged in vacuum. A lower concentration of CO_2 balanced with N_2 could be used to eliminate this problem. Another concern associated with using high CO_2 MAP for RTE meats may be the sour aroma and sour taste produced by the high concentration CO_2 in MAP utilized in this study. Finally, irradiated off-odor has been a critical issue for irradiated RTE meat in both vacuum and modified atmosphere packaging and needs to be resolved to assure consumers-satisfaction with these products.

Future studies should include combining irradiation, high CO_2 MAP and other ingredients or methods to not only control *L. monocytogenes* in RTE meats, but to also mitigate product quality changes.

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Table 1--Absorbed irradiation doses by frankfurters and cooked pork chops packaged in vacuum or high CO_2 MAP (experiment 1)

Target dose (kGy)	Average surface dose (kGy)		Average maximum dose (kGy)		Overall average dose (kGy)	
	Franks	Pork chops	Franks	Pork chops	Franks	Pork chops
1.0	0.970	1.020	1.251	1.400	1.110	1.209
1.5	1.444	1.510	1.929	2.100	1.764	1.807
2.0	1.947	2.012	2.578	2.765	2.259	2.388

Table 2--Absorbed irradiation doses by frankfurters and cooked pork chops packaged in vacuum and high CO_2 MAP (experiment 2)

Target doses (kGy)	Average surface dose (kGy)		Average maximum dose (kGy)		Overall average dose (kGy)	
	Franks	Pork chops	Franks	Pork chops	Franks	Pork chops
1.0	0.971	1.037	1.250	1.328	1.110	1.182
2.0	1.943	2.015	2.507	2.652	2.225	2.333

Table 3—Mean of radiation D_{10}-values (kGy) of *L. monocytogenes* on frankfurters and pre-cooked pork chops packaged in vacuum or high CO_2 MAP

Product	Packaging	N	Mean D_{10}-value	Std. Deviation	SEM	P-value
Frankfurters	Vacuum	9	0.66	0.15	0.03	0.619
	MAP	9	0.70	0.25	0.05	
Pre-cooked pork chops	Vacuum	9	0.59	0.09	0.02	0.137
	MAP	9	0.57	0.08	0.02	

Table 4—The recovery of *L. monocytogenes* (log cfu /cm^2) in irradiated frankfurters packaged in vacuum or MAP after 24 or 48 hours of storage at 2-4ºC (Rep 1)

	Count (log cfu /cm^2) in vacuum packages[1]						Count (log cfu /cm^2) in MAP packages[1]					
Dose[3] (kGy)	**Day 1** (Irradiation day)	**SE[2]**	**Day 2** (24 hours)	**SE[2]**	**Day 3** (48 hours)	**SE[2]**	**Day 1** (Irradiation day)	**SE[2]**	**Day 2** (24 hours)	**SE[2]**	**Day 3** (48 hours)	**SE[2]**
0	3.71[a]	0.06	4.89[b]	0.16	5.35[b]	0.09	3.79[a]	0.04	5.21[b]	0.05	5.39[b]	0.05
1.0	3.17	0.08	3.42	0.08	3.33	0.29	3.22	0.18	3.62	0.16	3.40	0.01
1.5	2.51	0.23	2.26	0.08	2.27	0.09	2.43	0.09	2.43	0.05	2.41	0.26
2.0	1.12	0.04	0.99	0.08	0.93	0.11	2.00	0.50	1.81	0.21	1.89	0.16

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 5—The recovery of *L. monocytogenes* (log cfu /cm^2) in irradiated frankfurters packaged in vacuum or MAP after 24 or 48 hours of storage at 2-4ºC (Rep 2)

Dose[3] (kGy)	Count (log cfu /cm^2) in vacuum packages[1]						Count (log cfu /cm^2) in MAP packages[1]					
	Day 1 (Irradiation day)	SE[2]	Day 2 (24 hours)	SE[2]	Day 3 (48 hours)	SE[2]	Day 1 (Irradiation day)	SE[2]	Day 2 (24 hours)	SE[2]	Day 3 (48 hours)	SE[2]
0	4.65	0.07	4.70	0.08	4.66	0.08	4.87	0.05	4.79	0.16	4.91	0.07
1.0	3.63	0.15	3.64	0.19	3.14	0.09	3.32	0.11	3.32	0.02	3.38	0.01
1.5	2.66	0.17	2.89	0.20	2.54	0.09	2.35	0.05	2.50	0.23	2.70	0.22
2.0	2.68[a]	0.13	1.38[b]	0.14	1.33[b]	0.03	2.05	0.53	2.03	0.38	1.53	0.06

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 6—The recovery of *L. monocytogenes* (log cfu /cm^2) in irradiated frankfurters packaged in vacuum or MAP after 24 or 48 hours of storage at 2-4ºC (Rep 3)

	Count (log cfu /cm^2) in vacuum packages[1]						Count (log cfu /cm^2) in MAP packages[1]					
Dose[3] (kGy)	**Day 1** (Irradiation day)	**SE[2]**	**Day 2** (24 hours)	**SE[2]**	**Day 3** (48 hours)	**SE[2]**	**Day 1** (Irradiation day)	**SE[2]**	**Day 2** (24 hours)	**SE[2]**	**Day 3** (48 hours)	**SE[2]**
0	4.48	0.08	4.84	0.04	4.74	0.02	4.77	0.02	4.59	0.17	4.90	0.07
1.0	3.36	0.02	3.66	0.07	3.36	0.12	3.64	0.06	3.37	0.06	3.38	0.08
1.5	2.43	0.01	2.45	0.11	2.87	0.24	2.63	0.13	2.50	0.08	2.59	0.12
2.0	1.49	0.05	1.67	0.37	1.37	0.14	1.43	0.01	1.28	0.09	1.53	0.12

[1] No significant difference between the means within same row ($p < 0.05$)
[2] Standard error of means
[3] The target irradiation dose

Table 7--Analysis of variance: Recovery of *L. monocytogenes* on irradiated cooked pork chops in vacuum or MAP packages after two days of storage at 2-4ºC

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	2247.632	29894.056	0.000	1.000
Pack	1	1.762	13.519	0.067	0.871
Time	2	0.465	23.043	0.006	0.920
Dose	3	122.454	508.020	0.000	0.996
Rep	2	0.075	2.167	0.935	0.994
Pack * Time	2	0.183	0.666	0.563	0.250
Pack * Dose	3	0.408	5.235	0.041	0.724
Time * Dose	6	0.076	0.347	0.898	0.148
Pack * Time * Dose	6	0.059	0.276	0.938	0.121
Pack * Rep	2	0.130	0.944	0.615	0.701
Time * Rep	4	0.020	0.072	0.986	0.090
Pack * Time * Rep	4	0.275	1.280	0.331	0.299
Dose * Rep	6	0.241	2.936	0.478	0.959
Pack * Dose * Rep	6	0.078	0.363	0.888	0.154
Time * Dose * Rep	12	0.219	1.019	0.487	0.505
Pack * Time * Dose * Rep	12	0.215	2.141	0.018	0.151

Table 8--Analysis of variance: The growth of *L. monocytogenes* on irradiated frankfurters in vacuum or MAP packages during refrigerated storage for 12 weeks

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	6872.171	919.403	0.001	0.998
Dose	3	642.940	174.725	0.000	0.989
Week	11	12.392	9.253	0.000	0.822
Pack	1	16.711	0.137	0.116	0.781
Rep	2	7.475	0.409	0.703	0.250
Dose * Week	33	1.977	1.801	0.021	0.474
Dose * Pack	3	5.316	3.699	0.081	0.649
Week * Pack	11	4.969	17.461	0.000	0.897
Dose * Week * Pack	33	1.305	.468	0.093	0.423
Dose * Rep	6	3.680	2.236	0.155	0.650
Week * Rep	22	1.339	0.817	0.680	0.452
Dose * Week * Rep	66	1.098	1.234	0.198	0.552
Pack * Rep	2	16.353	8.269	0.010	0.655
Dose * Pack * Rep	6	1.437	1.616	0.157	0.128
Week * Pack * Rep	22	1.430	1.608	0.072	0.349
Dose * Week * Pack * Rep	66	0.889	1.683	0.001	0.162

Table 9—The growth of *L. monocytogenes* (log cfu /cm^2) on irradiated frankfurters packaged in vacuum during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	5.04^{a,b,c} ± 0.14	4.94^{a,b,c} ± 0.03	4.75^{b} ± 0.07	4.73^{a,b} ± 0.05	4.76^{a,b} ± 0.06	4.91^{a,b,c} ± 0.64	4.48^{a,b} ± 0.02	5.00^{a,b,c} ± 0.15	5.72^{c,d} ± 0.16	6.07^{d} ± 0.10	5.65^{c,d} ± 0.48	6.10^{d} ± 0.08
1.0	3.55^{a,b,c} ± 0.10	3.27^{a} ± 0.10	2.86^{a} ± 0.11	2.65^{a} ± 0.04	2.88^{a} ± 0.26	2.66^{a} ± 0.07	3.38^{a,b} ± 0.25	3.06^{a,b} ± 0.54	3.99^{a,b,c} ± 0.37	5.23^{b,c} ± 0.71	5.75^{c} ± 1.03	4.51^{a,b,c} ± 0.46
1.5	2.43^{a,b} ± 0.24	1.81^{a} ± 0.18	1.45^{a} ± 0.02	1.85^{a} ± 0.48	1.17^{a} ± 0.19	1.03^{a} ± 0.04	1.90^{a} ± 0.64	1.93^{a} ± 0.51	3.03^{b,c} ± 0.50	4.23^{b,c} ± 0.96	4.63^{b,c} ± 0.19	4.79^{c} ± 0.36
2.0	1.06 ± 0.23	1.36 ± 0.36	0.36 ± 0.18	0.67 ± 0.45	0.94 ± 0.49	0.97 ± 0.41	0.45 ± 0.45	-0.23 ± 0.39	1.46 ± 0.41	0.46 ± 0.89	1.05 ± 0.31	0.23 ± 0.23

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 10—The growth of *L. monocytogenes* (log cfu /cm^2) on irradiated frankfurters packaged in high CO_2 MAP during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	4.82^{a,c}	4.80^{a,b,c}	4.87^{a,b,c}	5.13^{b}	4.63^{a,b,c}	4.56^{a,c}	5.05^{b,c}	4.58^{c}	4.59^{a,b,c}	4.35^{a}	4.60^{a,b,c}	3.77^{d}
	± 0.06	± 0.12	± 0.06	± 0.08	± 0.10	± 0.02	± 0.03	± 0.01	± 0.09	± 0.03	± 0.08	± 0.29
1.0	3.04	3.21	3.04	2.62	2.51	2.95	2.64	2.36	2.98	2.38	2.64	2.30
	± 0.09	± 0.14	± 0.13	± 0.06	± 0.07	± 0.19	± 0.04	± 0.21	± 0.62	± 0.24	± 0.16	± 0.17
1.5	2.19^{a}	1.78^{a,b}	2.06^{a}	2.11^{a}	1.82^{a,b}	1.64^{a,b}	1.45^{a,b}	0.39^{b}	1.57^{a,b}	1.86^{a,b}	2.05^{a}	1.60^{a,b}
	± 0.06	± 0.08	± 0.21	± 0.33	± 0.32	± 0.27	± 0.23	± 0.73	± 0.29	± 0.08	± 0.29	± 0.12
2.0	1.27	1.44	1.25	1.51	0.80	1.09	0.94	0.28	-0.33	0.49	0.01	0.87
	± 0.32	± 0.20	± 0.21	± 0.08	± 0.05	± 0.37	± 0.12	± 0.28	± 0.33	± 0.28	± 1.03	± 0.13

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 11—The growth of *L. monocytogenes* (log cfu /cm^2) on irradiated frankfurters packaged in vacuum during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	4.51^{a,b,c} ± 0.16	4.86^{a,d} ± 0.11	4.24^{b} ± 0.03	4.48^{b,c} ± 0.00	4.78^{a,c} ± 0.05	4.91^{a,d} ± 0.07	5.25^{d,e} ± 0.07	5.51^{e} ± 0.05	5.56^{e} ± 0.05	5.62^{e} ± 0.10	5.65^{e} ± 0.01	6.12^{f} ± 0.09
1.0	2.89^{a,b} ± 0.23	2.76^{a,b} ± 0.07	2.51^{a,b} ± 0.17	1.44^{b} ± 1.23	2.54^{a,b} ± 0.16	3.19^{a,b} ± 0.28	4.17^{a,c} ± 0.16	4.39^{a,c} ± 0.32	4.78^{a,c} ± 0.13	5.76^{c} ± 0.62	3.96^{a,c} ± 0.73	6.06^{c} ± 0.08
1.5	1.56^{a,b} ± 0.18	1.76^{a,b} ± 0.18	1.33^{a,b} ± 0.20	0.43^{b} ± 0.72	1.10^{b} ± 0.10	0.53^{b} ± 0.79	2.23^{a,b} ± 0.62	2.95^{a,b,c} ± 0.83	2.49^{a,b} ± 1.75	3.09^{a,b,c} ± 0.20	6.17^{c} ± 0.12	4.81^{a,c} ± 0.75
2.0	-0.17 ± 0.44	0.43 ± 0.13	-0.10 ± 0.49	0.50 ± 0.10	0.00 ± 0.00	0.10 ± 0.10	-1.00 ± 0.00	-0.20 ± 0.10	-0.51 ± 0.49	0.57 ± 0.99	2.83 ± 2.14	1.84 ± 0.06

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 12—The growth of *L. monocytogenes* (log cfu /cm^2) on irradiated frankfurters packaged in high CO_2 MAP during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	4.83 ± 0.16	4.92 ± 0.04	4.91 ± 0.14	4.94 ± 0.12	4.96 ± 0.11	4.81 ± 0.15	4.65 ± 0.04	4.91 ± 0.08	5.50 ± 0.30	4.92 ± 0.08	4.90 ± 0.32	4.58 ± 0.16
1.0	$3.19^{a,b,d}$ ± 0.27	$3.94^{a,b,d}$ ± 0.14	$2.72^{a,b}$ ± 0.16	$3.30^{a,d}$ ± 0.15	$2.67^{a,b}$ ± 0.03	$2.91^{a,b,d}$ ± 0.14	$2.32^{b,c}$ ± 0.08	$2.66^{a,b}$ ± 0.22	$2.67^{a,b}$ ± 0.18	$2.46^{a,b}$ ± 0.26	1.43^{c} ± 0.22	3.72^{d} ± 0.03
1.5	$2.48^{a,b}$ ± 0.09	$2.12^{a,b}$ ± 0.11	$2.02^{a,b}$ ± 0.11	$2.02^{a,b}$ ± 0.23	$1.94^{a,b}$ ± 0.09	$2.11^{a,b}$ ± 0.09	$1.62^{a,b}$ ± 0.16	$1.73^{a,b}$ ± 0.25	$1.88^{a,b}$ ± 0.20	$1.79^{a,b}$ ± 0.44	2.69^{a} ± 0.47	1.34^{b} ± 0.10
2.0	0.63^{a} ± 0.03	0.96^{a} ± 0.10	$0.32^{a,b}$ ± 0.16	0.55^{a} ± 0.25	0.72^{a} ± 0.12	0.79^{a} ± 0.15	0.38^{a} ± 0.24	0.67^{a} ± 0.24	0.39^{a} ± 0.21	0.44^{a} ± 0.25	-0.67^{b} ± 0.33	$0.20^{a,b}$ ± 0.10

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 13—The growth of *L. monocytogenes* (log cfu /cm^2) on irradiated frankfurters packaged in vacuum during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	4.71^{a,b} ± 0.12	4.72^{a,b} ± 0.07	4.59^{a,b} ± 0.06	5.21^{b,c} ± 0.01	5.12^{b,c} ± 0.16	5.18^{b,c} ± 0.14	5.48^{c} ± 0.19	5.48^{c} ± 0.06	5.78^{c} ± 0.05	5.47^{c} ± 0.29	5.79^{c} ± 0.19	5.76^{c} ± 0.06
1.0	3.15^{a,b} ± 0.10	3.21^{a,b} ± 0.02	2.40^{b} ± 0.10	2.63^{b} ± 0.18	3.41^{a,b,c} ± 0.28	3.41^{a,b,c} ± 0.05	4.29^{a,c} ± 0.53	4.43^{c} ± 0.11	4.51^{c} ± 0.01	5.87^{d} ± 0.47	5.78^{d} ± 0.12	6.39^{d} ± 0.04
1.5	1.63^{a,b} ± 0.20	1.90^{a,b} ± 0.03	0.69^{b} ± 0.85	1.64^{a,b} ± 0.11	1.99^{a,b} ± 0.05	2.55^{a} ± 0.28	2.45^{a} ± 0.22	4.29^{c} ± 0.07	5.59^{c,d} ± 0.12	5.92^{c,d} ± 0.38	6.01^{d} ± 0.41	5.45^{c,d} ± 0.28
2.0	0.63^{a} ± 0.25	0.70^{a,b} ± 0.35	0.36^{a,b} ± 0.06	0.10^{b} ± 0.10	0.42^{a,b} ± 0.06	0.26^{a,b} ± 0.14	1.04^{a,b} ± 0.65	2.08^{a,b,c} ± 1.04	3.87^{c} ± 0.89	2.84^{a,b,c} ± 0.12	3.95^{c,d} ± 0.31	5.69^{d} ± 0.94

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 14—The growth of *L. monocytogenes* (log cfu /cm^2) on irradiated frankfurters packaged in high CO_2 MAP during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	5.54 ± 0.30	5.17 ± 0.10	4.70 ± 0.10	4.69 ± 0.03	4.77 ± 0.03	4.54 ± 0.22	3.40 ± 1.46	2.84 ± 1.92	3.07 ± 1.39	3.45 ± 1.61	4.59 ± 0.12	4.24 ± 0.16
1.0	2.98 ± 0.05	3.37 ± 0.06	2.95 ± 0.13	2.79 ± 0.14	2.63 ± 0.07	2.52 ± 0.14	3.23 ± 0.74	3.06 ± 0.87	3.54 ± 0.78	3.36 ± 0.67	2.08 ± 0.39	2.50 ± 0.10
1.5	2.19 ± 0.54	2.08 ± 0.09	1.83 ± 0.07	1.91 ± 0.07	1.84 ± 0.03	0.82 ± 0.43	1.99 ± 0.39	1.72 ± 0.73	1.75 ± 0.37	1.59 ± 0.45	1.43 ± 0.30	1.00 ± 0.00
2.0	1.22 ± 0.05	1.13 ± 0.16	0.83 ± 0.44	0.57 ± 0.13	0.66 ± 0.12	0.93 ± 0.33	-0.17 ± 0.83	0.53 ± 0.53	0.10 ± 0.59	0.58 ± 0.19	0.52 ± 0.04	0.49 ± 0.76

[1] No significant difference between the means within same row ($p < 0.05$)
[2] Standard error of means
[3] The target irradiation dose

Table 15--Analysis of variance: The growth of *L. monocytogenes* in irradiated cooked pork chops in vacuum or MAP packages during refrigerated storage for 12 weeks

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	10607.183	200.850	0.005	0.990
Dose	3	757.880	135.704	0.000	0.985
Week	11	16.635	2.380	0.040	0.543
Pack	1	769.666	58.842	0.017	0.967
Rep	2	52.811	2.900	0.182	0.626
Dose * Week	33	2.291	0.985	0.507	0.330
Dose * Pack	3	8.146	2.203	0.189	0.524
Week * Pack	11	31.043	9.555	0.000	0.827
Dose * Week * Pack	33	3.421	1.867	0.016	0.483
Dose * Rep	6	5.585	1.332	0.352	0.523
Week * Rep	2	6.989	.867	0.073	0.642
Dose * Week * Rep	66	2.327	.270	0.167	0.559
Pack * Rep	2	13.080	2.558	0.130	0.355
Dose * Pack * Rep	6	3.697	2.017	0.076	0.155
Week * Pack * Rep	22	3.249	1.773	0.039	0.371
Dose * Week * Pack * Rep	66	1.833	1.571	0.004	0.153

Table 16—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops packaged in vacuum during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	5.23^{a} ± 0.09	5.81^{a,b} ± 0.17	5.22^{a} ± 0.02	6.38^{a,b,c} ± 0.41	6.35^{a,b,c} ± 0.44	7.29^{b,c} ± 0.29	6.81^{a,b,c} ± 0.31	6.68^{a,b,c} ± 0.35	6.31^{a,b,c} ± 0.57	7.76^{c} ± 0.09	7.60^{c} ± 0.14	7.92^{c} ± 0.58
1.0	3.42^{a} ± 0.08	3.61^{a} ± 0.09	3.66^{a} ± 0.20	3.51^{a} ± 0.21	3.50^{a} ± 0.23	5.62^{a,c} ± 0.28	3.79^{a} ± 0.53	3.53^{a} ± 0.77	4.82^{a} ± 0.62	5.05^{a,c} ± 0.88	8.08^{b} ± 0.18	7.29^{c} ± 0.40
1.5	2.34^{a} ± 0.43	2.99^{a,b,c} ± 0.58	2.50^{a,b} ± 0.03	3.93^{a,b,c} ± 0.57	3.90^{a,b,c} ± 0.60	3.98^{a,b,c} ± 0.25	3.02^{a,b,c} ± 0.42	3.02^{a,b,c} ± 0.23	3.13^{a,b,c} ± 0.52	5.24^{b,c} ± 0.42	5.68^{c,d} ± 0.76	7.33^{b,d} ± 1.07
2.0	1.67 ± 0.46	1.30 ± 0.42	1.48 ± 0.47	3.61 ± 0.90	3.59 ± 0.88	2.26 ± 0.48	2.64 ± 0.37	1.84 ± 0.70	0.20 ± 0.20	3.52 ± 1.80	4.66 ± 2.36	4.29 ± 2.15

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 17—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops packaged in high CO_2 MAP during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	5.27^{a,c} ± 0.05	5.48^{a,b,d} ± 0.04	6.05^{b} ± 0.18	5.15^{a,c} ± 0.03	5.16^{a,c} ± 0.04	5.22^{a,c} ± 0.02	5.14^{a,c} ± 0.04	5.08^{a,c} ± 0.08	5.31^{a,c} ± 0.34	5.57^{b,c,d} ± 0.06	4.90^{a} ± 0.10	6.30^{d} ± 0.36
1.0	3.68 ± 0.04	3.53 ± 0.17	3.40 ± 0.25	3.23 ± 0.08	3.20 ± 0.07	2.99 ± 0.09	2.68 ± 0.24	3.01 ± 0.03	3.10 ± 0.15	2.53 ± 0.46	2.05 ± 1.03	3.80 ± 0.26
1.5	2.90 ± 0.29	3.17 ± 0.39	2.68 ± 0.82	2.22 ± 0.09	2.24 ± 0.08	2.01 ± 0.09	1.64 ± 0.08	1.37 ± 0.31	1.53 ± 0.12	1.38 ± 0.69	2.67 ± 0.12	2.61 ± 0.47
2.0	1.73^{a,b} ± 0.09	1.44^{a,b,c} ± 0.16	1.57^{a,b} ± 0.23	2.05^{a} ± 0.34	2.07^{a} ± 0.32	1.12^{a,b,c} ± 0.18	0.74^{b} ± 0.37	1.17^{a,b,c} ± 0.30	0.20^{c} ± 0.20	1.67^{a} ± 0.24	0.56^{b} ± 0.31	0.86^{a,b,c} ± 0.14

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 18—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops packaged in vacuum during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	6.02[a] ± 0.27	6.36[a,b] ± 0.13	7.02[a,b] ± 0.85	7.21[a,b] ± 0.51	7.75[b] ± 0.05	7.54[a,b] ± 0.24	7.72[b] ± 0.08	7.62[a,b] ± 0.26	7.89[b] ± 0.12	8.00[b] ± 0.14	7.80[b] ± 0.09	7.67[a,b] ± 0.28
1.0	3.67[a] ± 0.26	3.37[a] ± 0.07	3.21[a] ± 0.13	4.06[a,b] ± 0.77	4.20[a,b] ± 0.62	4.52[a,b] ± 0.34	5.75[b,c] ± 0.50	7.33[c] ± 0.48	7.16[c] ± 0.18	7.15[c] ± 0.20	6.64[c] ± 0.05	4.13[a,b] ± 0.13
1.5	2.05 ± 0.15	2.10 ± 0.03	1.99 ± 0.16	1.85 ± 0.45	2.09 ± 0.30	3.41 ± 0.15	4.78 ± 0.96	5.09 ± 1.60	5.52 ± 1.33	5.54 ± 1.29	6.54 ± 1.51	5.54 ± 1.84
2.0	2.33[a,b] ± 0.50	0.72[a,b] ± 0.33	0.50[b] ± 0.26	1.03[a,b] ± 0.14	1.21[a,b] ± 0.30	2.05[a,b] ± 1.09	3.01[a,b] ± 0.71	2.78[a,b] ± 1.32	3.25[a,b] ± 1.69	3.21[a,b] ± 1.70	4.94[a,b] ± 1.46	5.77[a] ± 0.93

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 19—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops packaged in high CO_2 MAP during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	5.69[a] ± 0.22	5.29[a] ± 0.04	5.04[a] ± 0.02	5.43[a] ± 0.11	5.55[a] ± 0.04	3.22[a] ± 0.95	0.48[b] ± 0.25	0.26[b] ± 0.14	0.10[b] ± 0.10	3.83[a] ± 0.72	5.60[a] ± 0.39	5.25[a] ± 0.17
1.0	2.96[a,b,c] ± 0.12	3.47[b] ± 0.06	2.95[a,b,c] ± 0.08	3.21[c] ± 0.13b	3.28[b] ± 0.06	3.12[a,b,c] ± 0.10	2.91[a,b,c] ± 0.06	3.23[b,c] ± 0.08	3.04[a,b,c] ± 0.02	3.00[a,b,c] ± 0.04	2.54[a] ± 0.30	2.60[a,c] ± 0.17
1.5	2.49[a] ± 0.08	2.24[a,b] ± 0.20	2.34[a,b] ± 0.09	1.84[a,b] ± 0.10	1.81[a,b] ± 0.41	1.85[a,b] ± 0.32	1.62[a,b] ± 0.14	1.62[a,b] ± 0.08	2.73[a] ± 0.22	2.70[a] ± 0.24	0.66[b] ± 0.12	2.49[a] ± 0.95
2.0	1.50[a] ± 0.25	1.20[a,b,c] ± 0.17	1.17[a,b,c] ± 0.09	0.98[a,b,c,d] ± 0.10	1.25[a,b] ± 0.11	1.11[a,b,c] ± 0.14	0.48[b,c,d,e] ± 0.25	0.26[cd] ± 0.14	0.10[de] ± 0.10	-0.23[e] ± 0.39	0.52[b,d] ± 0.04	0.32[b,d,e] ± 0.16

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 20—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops packaged in vacuum during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	4.48 ± 0.12	5.20 ± 0.29	6.80 ± 0.46	6.65 ± 0.82	7.16 ± 0.13	6.69 ± 0.66	5.65 ± 0.20	7.52 ± 0.05	6.51 ± 0.36	4.29 ± 2.64	6.86 ± 0.51	4.23 ± 2.61
1.0	1.81^{a} ± 0.26	2.24^{a} ± 0.11	3.04^{a,b} ± 0.19	3.68^{a,b} ± 0.34	4.30^{a,b} ± 0.41	3.84^{a,b} ± 0.68	7.26^{b} ± 0.24	7.85^{b} ± 0.45	7.93^{b} ± 0.00	7.59^{b} ± 0.20	8.08^{b} ± 0.16	4.79^{a,b} ± 2.89
1.5	1.41^{a} ± 0.14	1.59^{a} ± 0.06	1.60^{a} ± 0.33	3.32^{a} ± 0.44	3.01^{a} ± 0.77	3.16^{a} ± 0.64	6.37^{b} ± 0.73	6.82^{b} ± 0.64	6.90^{b} ± 0.12	7.59^{b} ± 0.12	7.79^{b} ± 0.07	-1.00^{c} ± 0.00
2.0	0.36 ± 0.18	0.16 ± 0.16	-0.05 ± 0.53	0.42 ± 1.42	0.73 ± 1.04	1.79 ± 0.70	-0.28 ± 0.72	0.90 ± 0.36	3.83 ± 2.42	2.51 ± 2.16	2.67 ± 1.85	3.44 ± 1.89

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 21—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops packaged in high CO_2 MAP during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]	Mean[1] ± SE[2]
0	4.30[a,b] ± 0.02	4.25[a,b] ± 0.10	4.38[a,b] ± 0.06	3.76[a] ± 0.39	4.21[a,b] ± 0.02	4.41[a,b] ± 0.16	4.44[a,b] ± 0.06	4.29[a,b] ± 0.16	4.82[a,b] ± 0.21	3.83[a,b] ± 0.41	5.14[b] ± 0.47	3.87[a,b] ± 0.67
1.0	2.41 ± 0.28	2.04 ± 0.32	2.07 ± 0.13	1.81 ± 0.25	2.18 ± 0.35	2.41 ± 0.10	2.29 ± 0.17	1.90 ± 0.15	1.16 ± 1.09	2.61 ± 0.43	2.05 ± 0.30	1.84 ± 0.42
1.5	0.87 ± 0.36	1.10 ± 0.28	0.56 ± 0.14	0.96 ± 0.15	1.52 ± 0.26	1.42 ± 0.04	1.36 ± 0.14	1.69 ± 0.39	1.43 ± 0.13	0.30 ± 0.00	1.01 ± 0.17	0.30 ± 0.67
2.0	0.89[a] ± 0.15	0.70[a] ± 0.00	-0.13[a,b] ± 0.47	0.32[a,b] ± 0.16	-0.23[a,b] ± 0.39	0.36[a] ± 0.18	0.43[a] ± 0.22	0.20[a,b] ± 0.20	-1.00[b] ± 0.00	-0.23[a,b] ± 0.39	-0.20[a,b] ± 0.40	-1.00[b] ± 0.00

[1] Mean values within same row with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 22--Analysis of variance: The growth of *L. monocytogenes* on irradiated frankfurters in vacuum or MAP packages at room temperature (25 ºC) for 48 hours

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	953.471	634.986	0.002	0.997
Dose	3	104.350	149.048	0.000	0.987
Temp	1	3.881	3.691	0.195	0.649
Pack	1	2.834	10.314	0.085	0.838
Rep	2	1.502	0.898	0.475	0.305
Dose * Temp	3	0.221	1.053	0.435	0.345
Dose * Pack	3	0.082	1.047	0.438	0.344
Temp * Pack	1	0.338	4.436	0.170	0.689
Dose * Temp * Pack	3	0.366	32.403	0.000	0.942
Dose * Rep	6	0.700	2.522	0.101	0.623
Temp * Rep	2	1.051	3.821	0.073	0.510
Dose * Temp * Rep	6	0.210	18.584	0.001	0.949
Pack * Rep	2	0.275	1.912	0.238	0.423
Dose * Pack * Rep	6	0.079	6.962	0.016	0.874
Temp * Pack * Rep	2	0.076	6.743	0.029	0.692
Dose * Temp * Pack * Rep	6	0.011	0.185	0.980	0.011

Table 23--Analysis of variance: The growth of *L. monocytogenes* on irradiated cooked pork chops in vacuum or MAP packages at room temperature (25 ºC) for 48 hours

Source	df	Mean Square	F	p-value	Partial Eta Squared
Intercept	1	1804.409	6833.947	0.000	1.000
Dose	3	166.444	62.505	0.000	0.969
Temp	1	60.762	2.772	0.238	0.581
Pack	1	21.824	8.928	0.096	0.817
Rep	2	0.264	0.012	0.989	0.011
Dose * Temp	3	3.303	2.073	0.205	0.509
Dose * Pack	3	3.739	1.795	0.248	0.473
Temp * Pack	1	15.223	8.644	0.099	0.812
Dose * Temp * Pack	3	2.196	1.943	0.224	0.493
Dose * Rep	6	2.663	1.046	0.494	0.568
Temp * Rep	2	21.917	9.853	0.076	0.897
Dose * Temp * Rep	6	1.594	1.410	0.344	0.585
Pack * Rep	2	2.444	0.901	0.495	0.378
Dose * Pack * Rep	6	2.083	1.843	0.238	0.648
Temp * Pack * Rep	2	1.761	1.558	0.285	0.342
Dose * Temp * Pack * Rep	6	1.130	0.929	0.478	0.055

Table 24—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops at 25ºC for 48 hours (Rep 1)

Dose[3] (kGy)	Count (log cfu /g) in vacuum packages				Count (log cfu /g) in MAP packages			
	Mean[1] (4 ºC)	SE[2]	Mean[1] (25ºC)	SE[2]	Mean[1] (4 ºC)	SE[2]	Mean[1] (25ºC)	SE[2]
0	5.81	0.17	6.14	0.09	5.49	0.04	5.91	0.16
1.0	3.08	0.54	4.10	0.69	3.53	0.17	3.59	0.23
1.5	2.99	0.58	2.73	1.29	3.17	0.39	2.31	0.10
2.0	1.30	0.42	2.07	0.75	1.43	0.28	1.25	0.03

[1] No significant difference of means within the same row of the same packaging type ($p < 0.05$)
[2] Standard error of means
[3] The target irradiation dose

Table 25—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops at 25ºC for 48 hours (Rep 2)

Dose[3] (kGy)	Count (log cfu /g) in vacuum packages				Count (log cfu /g) in MAP packages			
	Mean[1] (4 ºC)	SE[2]	Mean[1] (25ºC)	SE[2]	Mean[1] (4 ºC)	SE[2]	Mean[1] (25ºC)	SE[2]
0	6.36[a]	0.13	8.68[b]	0.11	5.29	0.04	5.91	0.07
1.0	2.90[a]	0.48	5.48[b]	0.17	3.47	0.06	3.18	0.20
1.5	2.10[a]	0.03	4.69[b]	1.01	2.24	0.20	2.13	0.25
2.0	0.72	0.33	1.85	0.10	1.07	0.17	1.16	0.20

[1] Mean values within the same row of the same packaging type with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 26—The growth of *L. monocytogenes* (log cfu /gram) on irradiated cooked pork chops at 25ºC for 48 hours (Rep 3)

Dose[3] (kGy)	Count (log cfu /g) in vacuum packages				Count (log cfu /g) in MAP packages			
	Mean[1] (4 ºC)	SE[2]	Mean[1] (25ºC)	SE[2]	Mean[1] (4 ºC)	SE[2]	Mean[1] (25ºC)	SE[2]
0	5.20	0.29	9.88	0.10	4.25	0.10	9.07	0.22
1.0	1.85	0.39	5.94	1.93	2.04	0.32	2.17	0.09
1.5	1.59	0.05	5.16	1.60	1.10	0.29	1.44	0.28
2.0	0.16	0.16	2.12	2.12	0.70	0.00	3.57	1.80

[1] No significant difference of means within the same row of the same packaging type ($p < 0.05$)
[2] Standard error of means
[3] The target irradiation dose

Table 27-- The exterior lightness color value (L*) of frankfurters irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	Vacuum				MAP			
	Day 1		Day 28		Day 1		Day 28	
	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]
0	67.02[a]	0.27	66.55[a]	0.32	51.48[b]	0.25	66.55[a]	0.20
1.0	66.57[a]	0.56	66.79[a]	0.31	51.57[b]	0.17	66.55[a]	0.17
2.0	67.22[a]	0.36	66.42[a]	0.45	51.43[b]	0.24	66.79[a]	0.35

[1] Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 28-- The exterior red-green color value (a*) of frankfurters irradiated in vacuum and high CO_2 MAP packages

Dose [3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
0	23.84^{a} ± 0.21	22.48^{a} ± 0.47	22.70^{a} ± 1.18	22.57^{a,b} ± 0.74	24.50^{a} ± 0.32	23.63^{a,b} ± 0.29	22.93^{a,b} ± 0.25	22.57^{b} ± 0.33	21.56^{a} ± 0.67	20.92^{a,b} ± 0.57	21.03^{a} ± 0.80	20.59^{a,b} ± 0.27
1.0	22.88^{a} ± 0.31	22.61^{a} ± 0.31	22.84^{a} ± 0.65	22.37^{a,b} ± 0.62	23.62^{a} ± 0.41	22.92^{a} ± 0.47	22.86^{a} ± 0.42	22.70^{a,b} ± 0.30	23.63^{a} ± 0.96	20.78^{b} ± 0.21	21.84^{a,b} ± 0.35	20.59^{b} ± 0.34
2.0	22.77^{a} ± 0.41	21.84^{a} ± 0.91	22.53^{a} ± 0.67	21.31^{b} ± 0.53	23.76^{a} ± 0.18	22.82^{a,c} ± 0.27	21.49^{c} ± 0.46	21.56^{b,c} ± 0.70	21.10^{a} ± 0.35	21.80^{a} ± 0.58	21.42^{a} ± 0.58	19.18^{a,b} ± 0.59

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$) within the same replication.
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 29-- The interior red-green color value (a*) of frankfurters irradiated in vacuum and high CO_2 MAP packages

Dose [3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
0	17.86 ± 0.24	18.06 ± 0.26	17.44 ± 0.23	17.47 ± 0.25	17.80 ± 0.11	17.63 ± 0.11	17.77 ± 0.07	17.34 ± 0.15	$16.31^{a,b}$ ± 0.11	16.51^{a} ± 0.12	$15.91^{b,c}$ ± 0.12	$16.46^{a,b}$ ± 0.09
1.0	17.60 ± 0.17	18.02 ± 0.08	17.35 ± 0.17	17.94 ± 0.10	17.84 ± 0.15	17.47 ± 0.15	17.17 ± 0.13	17.48 ± 0.13	$16.09^{a,b}$ ± 0.07	16.63^{a} ± 0.14	$15.73^{b,c}$ ± 0.16	16.50^{a} ± 0.05
2.0	17.34 ± 0.15	17.64 ± 0.12	17.23 ± 0.19	17.06 ± 0.20	17.52 ± 0.17	17.39 ± 0.25	17.57 ± 0.12	17.45 ± 0.19	$15.89^{a,c}$ ± 0.10	16.67^{b} ± 0.10	$15.91^{b,c}$ ± 0.14	$16.37^{a,b}$ ± 0.15

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$) within the same replication.
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 30-- The lightness color value (L*) of cooked pork chops irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	76.77[a] ± 0.55	69.98[a] ± 1.70	71.86[a] ± 2.82	70.77[a] ± 1.15	73.40 ± 0.41	76.20 ± 1.14	73.24 ± 1.56	73.50 ± 0.52	68.89 ± 2.31	67.77 ± 1.25	66.97 ± 1.29	68.05 ± 1.68
1.0	74.73[a,b] ± 1.82	68.25[b] ± 1.50	69.70[a,b] ± 1.42	75.59[a] ± 1.25	75.15 ± 0.50	75.02 ± 0.71	71.00 ± 1.83	73.81 ± 1.44	68.07 ± 0.97	69.59 ± 0.91	73.04 ± 1.48	70.64 ± 0.81
2.0	78.90[a] ± 0.71	76.02[a,b] ± 1.11	77.57[a] ± 0.37	70.27[b] ± 1.30	74.80 ± 1.09	74.49 ± 1.57	72.02 ± 1.13	74.51 ± 1.16	69.43 ± 2.32	69.26 ± 1.26	70.88 ± 0.62	71.55 ± 0.48

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$) within the same replication.
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 31—The red-green color value (a*) of cooked pork chops irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	8.35[a] ± 0.25	10.29[b] ± 0.26	9.20[a,b] ± 0.79	9.68[a,b] ± 0.32	9.46[a] ± 0.14	8.83[a] ± 0.27	9.04[a] ± 0.34	8.97[a] ± 0.20	11.14[a] ± 0.43	11.21[a,c] ± 0.15	10.30[a] ± 0.22	10.08[a] ± 0.18
1.0	10.12[a] ± 0.58	10.88[a,b] ± 0.26	9.47[a,b] ± 0.37	8.18[b] ± 0.24	11.29[b] ± 0.22	9.60[a,b] ± 0.21	9.19[a] ± 0.38	8.32[a] ± 0.45	13.69[b] ± 0.53	11.57[c] ± 0.32	9.68[a] ± 0.40	9.65[a] ± 0.11
2.0	10.59[b] ± 0.26	10.43[a,b] ± 0.29	7.94[a] ± 0.10	9.54[a,b] ± 0.38	12.58[b] ± 0.39	9.81[a] ± 0.66	9.66[a] ± 0.21	8.27[a] ± 0.42	14.07[b] ± 0.77	11.48[c] ± 0.31	10.61[a,c] ± 0.29	9.24[a] ± 0.11

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$) within the same replication.
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 32—The blue-yellow color value (b*) of cooked pork chops irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	14.28^{a} ± 0.22	16.37^{b} ± 0.19	14.50^{a} ± 0.57	$15.62^{a,b,c}$ ± 0.18	15.56^{a} ± 0.18	$16.61^{a,b}$ ± 0.14	14.71^{a} ± 0.26	15.97^{a} ± 0.23	18.66^{a} ± 0.25	$17.73^{a,b}$ ± 0.07	$16.90^{b,c}$ ± 0.21	15.85^{c} ± 0.36
1.0	14.51^{a} ± 0.45	16.36^{b} ± 0.14	14.63^{a} ± 0.17	$14.33^{a,c}$ ± 0.26	14.82^{a} ± 0.22	17.24^{b} ± 0.30	14.99^{a} ± 0.28	$15.87^{a,b}$ ± 0.31	18.01^{a} ± 0.47	$18.12^{a,b}$ ± 0.23	$16.95^{a,b}$ ± 0.26	16.32^{c} ± 0.25
2.0	13.23^{a} ± 0.34	$15.39^{b,c}$ ± 0.64	13.10^{a} ± 0.24	15.19^{c} ± 0.28	14.14^{c} ± 0.29	17.17^{b} ± 0.49	$15.27^{a,c}$ ± 0.25	$16.34^{a,b}$ ± 0.37	17.13^{b} ± 0.57	17.22^{b} ± 0.18	17.27^{b} ± 0.20	$15.31^{a,c}$ ± 0.38

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$) within the same replication.
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 33--The pH of frankfurter irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
0	5.95[a] ± 0.00	5.72[b] ± 0.03	6.09[c] ± 0.01	5.89[a,d] ± 0.01	5.91[a] ± 0.06	5.85[a,b] ± 0.01	6.03[a] ± 0.03	5.86[a,b] ± 0.02	5.94[a] ± 0.01	5.79[b] ± 0.00	5.97[a,c] ± 0.02	5.80[b] ± 0.01
1.0	5.91[a] ± 0.03	5.75[b] ± 0.00	6.07[c] ± 0.01	5.85[a,d] ± 0.03	5.99[a] ± 0.01	5.83[b] ± 0.00	5.98[a,b] ± 0.01	5.85[a,b] ± 0.01	5.91[a] ± 0.01	5.78[b] ± 0.00	5.99[c] ± 0.01	5.81[b] ± 0.00
1.5	5.94[a] ± 0.01	5.78[b,d] ± 0.02	6.09[c] ± 0.01	5.81[d] ± 0.01	6.00[a] ± 0.03	5.81[b] ± 0.06	6.05[a] ± 0.02	5.84[b] ± 0.00	5.93[a] ± 0.00	5.80[b] ± 0.03	5.97[a,c] ± 0.01	5.79[b] ± 0.01

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$) within the same replication.
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 34--The pH of cooked pork chops irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	Vacuum				MAP			
	Day 1		Day 7		Day 1		Day 7	
	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]
0	6.29	0.08	6.29	0.02	6.33	0.02	6.40	0.06
1.0	6.31	0.15	6.36	0.01	6.39	0.09	6.37	0.05
2.0	6.25	0.05	6.39	0.02	6.38	0.01	6.45	0.06

[1] No significant difference between the means within same row and same column ($p < 0.05$)
[2] Standard error of means
[3] The target irradiation dose

Table 35--Purge (grams) of frankfurters irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	Vacuum				MAP			
	Day 1		Day 28		Day 1		Day 28	
	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]
0	3.88[a]	0.11	4.67[a]	0.08	0.96[b]	0.47	0.34[b]	0.03
1.0	3.44[a]	0.38	4.44[a]	0.01	0.79[b]	0.02	0.66[b]	0.27
2.0	2.92[a]	1.65	4.52[a]	0.15	0.85[b]	0.08	0.41[b]	0.05

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$).
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 36--Purge (grams) of cooked pork chops irradiated in vacuum and high CO_2 MAP packages

	Vacuum				MAP			
Dose[3] (kGy)	**Day 1**		**Day 7**		**Day 1**		**Day 7**	
	Mean[1]	**SE[2]**	**Mean[1]**	**SE[2]**	**Mean[1]**	**SE[2]**	**Mean[1]**	**SE[2]**
0	2.84[a]	0.31	3.22[a]	0.53	1.13[a,b]	0.66	1.20[a,b]	0.11
1.0	2.05[a,b]	0.50	3.35[a]	0.61	0.76[b]	0.07	1.09[a,b]	0.25
2.0	2.25[a,b]	0.10	3.54[a]	0.39	1.19[b]	0.48	1.43[b]	0.05

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$).
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 37--The TBA values of frankfurters irradiated in vacuum and high CO_2 MAP packages

Dose [3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
0	0.37^{a} ± 0.01	0.40^{a} ± 0.01	$0.40^{a,c}$ ± 0.00	0.47^{b} ± 0.02	0.40^{a} ± 0.01	0.41^{a} ± 0.00	0.49^{c} ± 0.01	0.48^{c} ± 0.01	0.64^{a} ± 0.03	0.63^{a} ± 0.01	0.60^{a} ± 0.01	$0.59^{a,c}$ ± 0.01
1.0	$0.38^{a,c}$ ± 0.01	0.37^{a} ± 0.01	0.42^{c} ± 0.00	$0.39^{a,c}$ ± 0.00	$0.41^{a,b}$ ± 0.02	$0.41^{a,b}$ ± 0.00	$0.46^{a,c}$ ± 0.01	0.40^{b} ± 0.01	0.49^{b} ± 0.00	$0.55^{a,b}$ ± 0.01	$0.55^{a,b}$ ± 0.00	$0.54^{a,b,c}$ ± 0.01
2.0	0.37^{a} ± 0.01	0.39^{a} ± 0.01	$0.41^{a,c}$ ± 0.01	0.38^{a} ± 0.00	0.38^{a} ± 0.01	$0.40^{a,b}$ ± 0.01	$0.44^{b,c}$ ± 0.02	0.45^{b} ± 0.01	$0.57^{a,c}$ ± 0.04	0.50^{a} ± 0.01	$0.52^{a,c}$ ± 0.03	0.61^{c} ± 0.01

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$) within the same replication.
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 38--The TBA values of cooked pork chops irradiated in vacuum and high CO_2 MAP packages

Dose[3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	0.41 ± 0.09	0.27 ± 0.04	0.27 ± 0.02	0.42 ± 0.02	0.11[a] ± 0.01	0.19[a] ± 0.03	0.14[a] ± 0.01	0.17[a] ± 0.02	0.21[a] ± 0.02	0.24[a] ± 0.03	0.20[a] ± 0.00	0.21[a] ± 0.02
1.0	0.21 ± 0.04	0.21 ± 0.02	0.18 ± 0.01	0.29 ± 0.09	0.07[a] ± 0.00	0.05[a] ± 0.00	0.13[a] ± 0.00	0.17[a] ± 0.01	0.21[a] ± 0.03	0.33[a,b] ± 0.05	0.17[a] ± 0.01	0.58[b] ± 0.02
2.0	0.32 ± 0.01	0.32 ± 0.02	0.20 ± 0.04	0.33 ± 0.10	0.36[b] ± 0.04	0.05[a] ± 0.01	0.12[a] ± 0.01	0.28[a] ± 0.08	0.18[a] ± 0.01	0.32[a] ± 0.06	0.20[a] ± 0.01	0.33[a] ± 0.07

[1]Mean values within same row and same column with different superscripts are statistically significantly different ($p < 0.05$) within the same replicati
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 40--LS means[1] ± standard errors for sensory attributes of unheated frankfurters irradiated at different levels

Dose (kGy)	Irradiated off-aroma[2]	Sour-like off-aroma[2]	Frankfurter aroma[2]
0	2.6 ± 0.7	1.9 ± 0.6	6.2 ± 0.7
1.0	3.9 ± 0.7	2.2 ± 0.6	5.5 ± 0.7
2.0	3.2 ± 0.7	2.4 ± 0.6	5.2 ± 0.7

[1]Data for packaging treatment were pooled since no interaction between packaging and irradiation effects was observed. There were no significant differences
between treatments for any of the attributes.
[2]Line scale, numerical value of 15; none=0; intense=15

Table 41—LS means[1,2] ± standard errors for sensory attributes of heated frankfurters packaged using different techniques

Packaging	Irradiated off-aroma[4]	Frankfurter aroma[4]	Denseness[5] (Appearance)	Firmness[6]	Irradiated off-flavor[4]	Sour-like aroma[4]	Frankfurter flavor[4]
Vacuum	$1.9^{a} \pm 0.2$	5.3 ± 0.7	$12.6^{a} \pm 0.4$	$11.0^{a} \pm 0.5$	1.4 ± 0.4	$1.4^{a} \pm 0.5$	$7.5^{a} \pm 0.9$
MAP[3]	$1.2^{b} \pm 0.2$	5.8 ± 0.7	$3.7^{b} \pm 0.4$	$4.9^{b} \pm 0.5$	1.5 ± 0.4	$4.0^{b} \pm 0.5$	$6.7^{b} \pm 0.9$

[1]Data for irradiation treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2] Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3]Modified atmosphere packaging.
[4] Line scale, numerical value of 15; none=0; intense =15.
[5]Line scale, numerical value of 15; not dense=0; very dense=15. Denseness was evaluated by appearance and not by mouth-feel.
[6] Line scale, numerical value of 15; not firm=0; very firm=15.

Table 42--LS Means[1,2] ± standard errors for sensory attributes of heated frankfurters irradiated at different levels

Doses(kGy)	Irradiated off-aroma[3]	Frankfurter aroma[3]	Denseness[4] (Appearance)	Firmness[5]	Irradiated off-flavor[3]	Sour-like aroma[3]	Frankfurter flavor[3]
0	$2.3^{a} \pm 0.3$	$4.3^{a} \pm 0.7$	$7.6^{a} \pm 0.5$	7.3 ± 0.5	1.6 ± 0.4	2.8 ± 0.5	$6.6^{a} \pm 0.9$
1.0	$0.8^{b} \pm 0.3$	$6.1^{b} \pm 0.7$	$8.4^{b} \pm 0.5$	8.2 ± 0.5	1.1 ± 0.4	2.3 ± 0.5	$7.6^{b} \pm 0.9$
2.0	$1.6^{ab} \pm 0.3$	$6.3^{b} \pm 0.7$	$8.5^{b} \pm 0.5$	8.3 ± 0.5	1.5 ± 0.4	2.9 ± 0.5	$7.0^{ab} \pm 0.9$

[1] Data for packaging treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2] Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3] Line scale, numerical value of 15; none=0, intense =15.
[4] Line scale, numerical value of 15; not dense=0, very dense=15.
[5] Line scale, numerical value of 15; not firm=0, very firm=15

Table 43—LS means[1,2] for sensory attributes of unheated cooked pork chops packaged using different techniques

Packaging	Irradiation off-aroma[4]	Sour-like aroma[4]	Unheated cooked pork chop aroma[4]
Vacuum	2.8[a]	1.5[a]	4.0[a]
MAP[3]	4.0[b]	2.3[b]	3.1[b]
SEM[5]	0.5	0.5	0.4

[1] Data for irradiation treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2] Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3] Modified atmosphere packaging.
[4] Line scale, numerical value of 15; none=0; intense =15.
[5] ± standard error of the mean.

Table 44—LS means[1,2] for sensory attributes of unheated cooked pork chops irradiated at different levels

Dose (kGy)	Irradiated off-aroma[3]	Sour-like Aroma[3]	Unheated cooked pork chop aroma[3]
0	2.2[a]	1.9	4.9[a]
1.0	3.8[b]	1.8	2.9[b]
2.0	4.4[b]	2.2	2.9[b]
SEM[4]	0.6	0.5	0.5

[1]Data for packaging treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2]Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3]Line scale, numerical value of 15; none=0; intense=15.
[4]± standard error of the mean

Table 45—LS means [1,2] for sensory attributes of unheated cooked chops packaged using different techniques and irradiated at different levels

Treatment	Pink[4]	Brown[4]
Control-vacuum	1.5[a]	8.0[b]
1.0 kGy-vacuum	4.5[b]	7.1[b]
2.0 kGy-Vacuum	8.4[c]	3.0[a]
Control-MAP[3]	1.1[a]	7.6[b]
1.0 kGy-MAP	1.3[a]	7.0[b]
2.0 kGy-MAP	1.8[a]	7.1[b]
SEM[5]	0.5	0.9

[1] An interaction was noted between the packaging and irradiation treatments. Individual treatment means are, therefore, reported.
[2] Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3] Modified atmosphere packaging.
[4] Line scale, numerical value of 15; none=0, intense =15.
[5] ± standard error of the mean.

Table 46—LS means[1,2] for sensory attributes of heated cooked pork chops packaged using different techniques

Packaging	Irradiated off-aroma[4]	Sour-like aroma[4]	Pork aroma[4]	Firmness[5]	Juiciness[6]	Irradiated off-flavor[4]	Sourness[4]	Pork flavor[4]
Vacuum	3.6	1.8	3.2	8.0[a]	3.0[a]	2.8	2.7[a]	3.9
MAP[3]	3.4	1.9	3.0	5.8[b]	4.9[b]	3.0	3.8[b]	3.3
SEM[7]	0.5	0.5	0.4	0.6	0.4	0.4	0.7	0.6

[1] Data for irradiation treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2] Means in a column followed by a different superscripts are significantly different ($p<0.05$).
[3] Modified atmosphere packaging
[4] Line scale, numerical value of 15; none=0; intense =15.
[5] Line scale, numerical value of 15; not firm=0; very firm=15.
[6] Line scale, numerical value of 15; not juicy=0; very juicy=15.
[7] ± standard error of the mean.

CHAPTER 4—CONTROL OF *SALMONELLA ENTERICA* TYPHIMURIUM AND *CAMPYLOBACTER JEJUNI* IN CHICKEN BREAST MEAT BY IRRADIATION COMBINED WITH MODIFIED ATMOSPHERE PACKAGING

A paper to be submitted to the Journal of Food Protection

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ABSTRACT

Salmonella and *Campylobacter* are leading causes of human foodborne illnesses originating from meat and poultry products. Cross contamination of "clean" products by contaminated products or equipment not only happens in processing plants, but can also occur in kitchens and refrigerators of individual families. Therefore, new intervention strategies are needed for meat and poultry products to better protect consumers from these pathogens. Vacuum or modified atmosphere packaging is a common packaging technique used by the meat and poultry industry to extend the shelf life of meat products. Irradiation has been well established as an antibacterial treatment to reduce pathogens on meat and poultry. Combining irradiation with high CO_2 + CO modified atmosphere

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packaging (MAP) was investigated in this study for control of *Salmonella enterica* Typhimurium and *Campylobacter jejuni* in chicken breast meat. The radiation sensitivity (D_{10}-value) of these two pathogens in chicken breast meat was the same in either vacuum or high CO_2 MAP. The D_{10}-values in vacuum or high CO_2 MAP packaging were 0.55 ± 0.03 kGy or 0.54 ± 0.03 kGy for *Salmonella* and 0.31 ± 0.01 kGy or 0.29 ± 0.03 kGy for *Campylobacter*. Both pathogens survived in both vacuum and high CO_2 MAP through 6 weeks of refrigerated storage. *Salmonella* Typhimurium grew in both vacuum and MAP when the product was exposed to room temperature. Carbon monoxide in high CO_2 MAP enhanced the red color of both irradiated and non-irradiated chicken breast meat. Irradiation is an effective means of eliminating *Salmonella* and *Campylobacter* from meat or poultry packaged in either vacuum or MAP and would reduce the chance of cross contamination in retail stores or home kitchens. However, irradiated off-odor and sour-aroma were observed for raw irradiated chicken meat packaged with either vacuum or modified atmosphere packaging, and additional means to mitigate quality changes may be necessary for these products.

Key words: *Salmonella*, *Campylobacter*, irradiation, modified atmosphere packaging, chicken breast meat

INTRODUCTION

Many studies have shown that *Salmonella enterica* and *Campylobacter* spp are most common causes of foodborne illnesses in the world. The human gastroenteritis caused by these pathogens has been frequently associated with the handling or consumption of contaminated poultry products *(53, 78)*. The poultry industry has been

striving to control the infection incidence of *Salmonella* and *Campylobacter* in live birds and to eliminate the contamination or cross contamination of these bacteria on poultry carcasses *(52, 76)*. Common practices on farms include applying antimicrobials in feed or water along with environmental hygiene. Post-harvest scalding sprays and carcass chilling with chlorinated water are used as intervention strategies in processing plants along with the Hazard Analysis and Critical Control Point System (HACCP) *(75, 74, 46, 52)*. While the current interventions have significantly reduced the consumer exposure to these food hazards, the industry has faced persistent or even increased frequency of positive samples of *Salmonella* among broilers as a result of US Food Safety and Inspection Service (USDA-FSIS) testing *(62, 5)*. Because the practical limit may have been reached in processing plants with the current methods used to reduce the contamination or cross contamination of *Salmonella* and *Campylobacter* on broilers, additional control measures are needed to further reduce the prevalence of *Salmonella* and *Campylobacter* on poultry products *(89, 76, 21, 70)*.

The use of ionizing radiation to inactivate *Salmonella, Campylobacter* and other foodborne pathogens in meat and poultry products has been well documented *(81, 66 65, 80, 45)*. However, irradiation has been reported to cause meat quality changes, such as off-odor, changed meat color and lipid oxidation *(50, 27, 61, 59)*. These quality changes have limited the consumer acceptance of commercialized irradiated fresh meat products *(55)*. To minimize these side effects of irradiation, many studies have been conducted on combination of other hurdles with irradiation to maintain both safety and quality of irradiated meat products. One of the approaches studied was the combination of irradiation with modified atmosphere packaging *(43)*.

Modified atmosphere packaging (MAP) with either low (20-30%) or high (60-100%) carbon dioxide content has been used to inhibit spoilage bacteria and to extend the shelf life of fresh meat and poultry *(83, 30, 67)*. It was also observed that high CO_2 MAP inhibited the growth of *E. coli O157:H7*, *Salmonella*, *Listeria monocytogenes* and *Campylobacter jejuni* in meat products *(20, 67)*. Some studies have combined irradiation with modified atmosphere packaging to control food-borne pathogens in the meat products. Patterson *(64)* reported that *Salmonella* Typhimurium and *E. coli O157:H7* in minced chicken meat were more sensitive to irradiation in MAP with 100% CO_2 than in air. However, Chiasson et al. *(13)* observed that when ground beef was packaged in MAP with 30% CO_2, 60% O_2 and 10% N_2, *E. coli O157:H7* and *Salmonella* Typhimurium were more sensitive to irradiation than in MAP containing 100% CO_2 or in vacuum packages. To avoid lipid oxidation induced by irradiation, packaging techniques that eliminate oxygen from meat or poultry products are preferred *(4)*. Therefore, when combined with irradiation, high CO_2 (low O_2) MAP might be a possible hurdle to improve control *Salmonella* and *Campylobacter* in poultry products, while minimizing quality changes. However, a high concentration of CO_2 in MAP can cause undesirable color change in meat and poultry products *(30, 11)*. Further, if MAP contains residual oxygen, fresh meat color will deteriorate even faster than in aerobic packaging during storage *(87)*.

Many studies have reported that irradiation increased redness of pork and poultry when products were packaged in vacuum *(60, 58)*. A previous study *(25)* also showed that irradiation made pork chops packaged in low O_2 MAP (with 25% CO_2 /75% N_2 or 50% CO_2/50% N_2) pinker than non-irradiated product; however, after 12 days storage,

the pink color of irradiated pork chops in MAP with 50% CO_2/50% N_2 faded, and the product became browner than non-irradiated product.

The Food and Drug Administration has approved carbon monoxide (0.4%) as a MAP gas *(84)*. Carbon monoxide reacts with meat myoglobin to produce bright cherry-red carboxymyoglobin with greater oxidative and color stability than oxymyoglobin *(48)*. Therefore, addition of CO into high CO_2 MAP might minimize the color deterioration in fresh meat and poultry caused by high concentration of CO_2 in MAP. Previous consumer tests *(88)* showed that pork loins packaged in 99% CO_2 / 1% CO had the most favorable color value compared with the product packaged in 100% CO_2, 100% O_2 or 100% CO. Few studies have been done to evaluate the effect of irradiation on bacterial and sensory quality of poultry products packaged in MAP with high concentration of CO_2 and low CO. Further, few studies have investigated the recovery or survival of *Salmonella or Campylobacter* on irradiated meat products in high CO_2 + CO packaging at refrigeration temperature or with temperature abuse.

The objective of this study was to test the hypothesis that irradiation combined with high CO_2 MAP + CO is at least as effective as irradiation with vacuum packaging for reducing *Salmonella enterica* Typhimurium or *Campylobacter jejuni* on fresh chicken breast meat, and for inhibiting the growth of survivors while providing superior color for retention of attractive fresh poultry appearance. Assessment of meat quality (color, oxidative rancidity, pH and package purge) and sensory evaluations of both raw and cooked products were included in this study.

MATERIALS AND METHODS

Experimental design. This study was conducted as two experiments. The microbiology assessment was done in experiment 1 while the product quality effects were evaluated in experiment 2. A random block design was used for both experiments. A 2 × 4 factorial design was used for treatments in experiment 1 to determine radiation D_{10}-values for *Salmonella enterica* Typhimurium and *Campylobacter jejuni* in chicken breast meat and to assess the survivor growth status during refrigerated storage and under the temperature abuse conditions. Vacuum and MAP comprised two levels of packaging while irradiation doses of 0 kGy (control), 0.5 kGy, 1.0 kGy and 1.5 kGy (for *Salmonella*) , or 0 kGy (control), 0.25 kGy, 0.50 kGy and 0.75 kGy (for *Campylobacter*) comprised four levels of irradiation dose. A 2 × 3 factorial design was used for sensory evaluation in experiment 2. Vacuum and MAP comprised two levels of packaging with irradiation doses of 0 kGy (control), 1.0 kGy and 1.5 kGy as three levels of irradiation dose. A 2 × 3 × 2 factorial design was used for color, purge, pH and rancidity evaluations. The two packaging treatments (vacuum and MAP), three irradiation doses (0 kGy, 1.0 kGy and 1.5 kGy) and two storage times (first day and the 7th day after irradiation) were used for these assessments. There were three samples measured for each treatment in experiment 1 and two samples for each treatment in experiment 2. Both experiments were repeated three times.

Experiment 1 was designed to determine and compare the radiation sensitivity (D_{10}-value) of Salmonella enterica Typhimurium or *Campylobacter jejuni in chicken breast meat packaged with either vacuum or high CO_2 MAP packaging, and to evaluate*

the fate of *survivors during storage at 2-4 ºC and following temperature abuse at room temperature (22-25 ºC).*

Preparation of meat samples. Refrigerated fresh chicken breasts (packaged in foam trays, 3-4 pieces /tray) were purchased from a local supplier. Individual chicken breasts were weighed and trimmed to about 100 grams per piece. Single pieces of chicken breast were placed into high barrier pouches (Curlon Grade 861, 3cc O_2 / 645 cm2 / 24 h at 23 ºC and 0% RH; Cryovac Division, W.R. Grace Co., Duncan, SC, U.S.A.). The chicken breasts for inoculation were immediately transferred to the Pathogen Laboratory in the Iowa State University Food Safety Research Laboratory (ISU-FSRL). The sample for quality evaluation (non-inoculated) was packaged in the Iowa State University Meat Laboratory.

Preparation of bacterial cultures. Four strains of *Salmonella enterica* Typhimurium (S-07, S-G25, S-G26 and S-G27) were supplied by the ISU-FSRL. Frozen stocks were separately transferred to 10 ml Tryptic Soy Broth (TSB) (Difco, Detroit, MI, U.S.A.) and incubated at 37 ºC for 24 hours. The cultures were streaked onto Tryptic Soy Agar (Difco) slants and incubated at 37 ºC for 24 hours. The slants were stored under refrigeration at the ISU-FSRL and used as stock for the three replications of the present study. A loop-full of each *Salmonella* culture was transferred into 10 ml TSB and incubated at 37 ºC for 24 hours. One milliliter of the broth culture was then individually transferred into 99 ml of TSB and incubated at 37 °C for 24 hour. The concentration of the bacteria reached about 8 log /ml. The inoculum was prepared by combining 2.5 ml of

each culture into 90 ml of peptone water. The cocktail contained approximately equal numbers of each strain with a total concentration of bacteria of 7 log / ml.

Five strains of *Campylobacter jejuni* (OA 11 #1, BF 8-11, BF 10-13, BF 10-7, OA 11 #32) were supplied by the Iowa State University Department of Veterinary Microbiology and Preventive Medicine. The procedures for preparing the inoculum were reported by Luo et al. *(49)*. Frozen stocks were transferred to 10 ml of Muella Hinton (MH) broth (BD Diagnostic Systems, Spark, MD, USA) and incubated at 42ºC for 3-7 days in a CO_2 incubator (Thermo Forma, Model 3130, Marietta, OH, USA). The atmosphere in the incubator was programmed and automatically adjusted to be 5% O_2, 10% CO_2 and 85% N_2. The cultures were streaked onto plates of Muella Hinton Agar (BD) with *Campylobacter* growth supplement and Preston *Campylobacter* selective supplement (Oxoid Ltd, Basingstoke, UK), and incubated at 42ºC for 48-72 hours in the CO_2 incubator. Characteristic colonies were transferred into 9 ml MH broth and incubated at 42ºC for 24-48 hours in the CO_2 incubator. One milliliter of each culture was then transferred into 99 ml MH broth and incubated at 42ºC for 24 hours in the CO_2 incubator. The average concentration of the cells for each strain reached 8 log /ml. Then, 20 ml of each culture was combined into a sterilized dilution bottle to make the five strain cocktail.

Inoculation and packaging. Two groups of chicken breast samples were separately inoculated with *Salmonella* or *Campylobacte*r. One milliliter of inoculum was placed on the chicken breast in each pouch with a sterilized pipette. The packages were manually massaged for about 1-2 min to distribute the inoculum evenly. The concentration of *Salmonella* on each piece of chicken breast meat was approximately 5

log /gram. The concentration of *Campylobacter* was approximately 6 log /gram. Pouches were immediately vacuum or MAP packaged with a Multivac (model A 300/52) packaging machine (Multivac Inc, Wolfertschwenden, Germany) in the FSRL. Cylinders with the desired gas mixture (99.5% CO2 and 0.5% CO) for MAP packaging were purchased from Linweld Co. (Linweld Co., Lincoln, NE, U.S.A.). The MAP packaging first applying vacuum (10-13 mbars), and then flushing the gas mixture into the pouches (pressure of 680-700 bars) with simultaneous sealing. The volume ratio of gas to the chicken breast meat in a single MAP package was about 4:1. After inoculation and packaging, samples were stored at 2-4 ºC for 12 hours before irradiation.

Irradiation. The inoculated, packaged samples were irradiated at the Iowa State University Linear Accelerator Facility (ISU-LAF). The electron beam irradiation was generated by a Circe-III linear electron accelerator at an energy level of 10 MeV and 10 kW (MeV Industries S.A., Jouy-Josas, Cedex, France). The target irradiation doses for chicken breast meat containing *Salmonella* were 0.5, 1.0 and 1.5 kGy; for samples inoculated with *Campylobacter* the target doses were 0.25, 0.50 and 0.75. Alanine pellet dosimeters (5 mm × 5 mm) (Broker Analytische Messtechnik, Rheinstetten, Germany) were placed on the top and bottom surface of sample pouches to measure the actual absorbed energy (dose). Immediately after irradiation, the absorbed doses were measured by electron paramagnetic resonance on a Broker EMS 104 EPR Analyzer. The average surface dose, overall average dose and average maximum doses absorbed by the chicken breast meat in vacuum and MAP are listed in Tables 1 and 2.

Following irradiation, the samples were stored at 2-4 ºC in the FSRL Pathogen Laboratory.

Determination of D_{10}-value. Plating of the organisms was conducted immediately after the irradiation. The packages of chicken breast meat were opened aseptically, and the meat was cut into small pieces with sterilized scissors, and then mixed and massaged manually from outside of the packages. Twenty-five grams of sample from each package was aseptically weighed into a sterile plastic stomacher bag (Whirl-Pack filter bag B01318, Nasco, Fort Atkinson, WI, U.S.A.) with 225 ml of peptone water (Difco, Becton Dickinson, Sparks, MD, U.S.A.), and homogenized in a Stomacher blender (Seward Stomacher Blender, Model 4000, Tekmar Co., Cincinnati, OH, U.S.A.) for 60 seconds at high speed. Aliquotes (0.1 ml) of the homogenate were surface plated onto XLD (Difco) plates for *Salmonella*. The homogenate of sample containing *Campylobacter* was surface plated onto Muella Hinton Agar (BD) plates with *Campylobacter* growth supplement and Preston *Campylobacter* selective supplement (Oxoid). The plates were incubated at 37 ºC for 24 hours for *Salmonella* and at 42 °C for 48 hours in the CO_2 incubator for *Campylobacter*. Radiation D_{10}-value of bacteria is defined as the amount of radiation energy (dose) needed to reduce 90% (cfu) of the target microorganism in irradiated food products. The D_{10}-value was determined by calculating the negative reciprocal of the slope of the regression line for the plot of log number of survivors ($\log_{10}$ cfu / gram) versus irradiation dose (kGy) (Clavero et al., 1994).

Enumeration of survivors during storage. Recovery of the pathogens on irradiated chicken breast meat was measured after 24 and 48 hours of storage at 2-4 ºC, and at one week intervals for six weeks to determine the fate of the survivors. For the temperature abuse test, samples were held for 7 days at 2-4 ºC followed by room

temperature (22-25 ºC) for 48 hours prior to enumeration. The plating method was the same as for the determination of D_{10}-values.

Experiment 2 was designed to determine and compare quality and sensory attributes of irradiated chicken breast meat packaged with vacuum or with high CO_2 + CO MAP packaging.

Packaging of meat samples. Uninoculated chicken breast meat (see experiment 1) was packaged in the ISU Meat Laboratory using a Multivac (model C500) packaging machine (Multivac Inc, Wolfertschwenden, Germany). The vacuum and gas packaging procedures were the same as in experiment 1.

Irradiation. Samples for quality and sensory assessment were irradiated at the same facility (ISU-LAF) but at a different time than the inoculated samples. The target doses for experiment 2 were 1.0 kGy and 1.5 kGy. The average surface dose, overall average dose and average maximum doses absorbed by the chicken breast meat in vacuum and MAP are listed in Table 3.

Following irradiation, the samples were stored at 2-4 ºC prior to quality evaluation. The samples for sensory evaluation were transferred to ISU Sensory Evaluation Center immediately following irradiation.

Color Measurement. CIE color values (L*, a* b*) of the surface color of the chicken breast meat were measured with a Hunter Lab LabScan (Model LS 1500, Hunter Associated Labratories Inc., Reston, VA, U.S.A.). CIE standard illuminant A

(incandescent or tungsten lamplight), 10 degree observer and a 1.75-inch port insert were used. The temperature of the light source was 2,856 ºK. Color of the chicken breast was measured on the packaged samples through the packaging material. Three readings were randomly collected from different locations on each sample. Measurements were conducted on day 1 and day 7 after irradiation.

Package Purge. After the color measurement, the same samples were used for determining package purge measurement. Purge was measured by first weighing the empty pouches before packaging, then weighing the sample with packaging material prior to opening the package. The sample was then removed, dried with paper towels and weighed. The quantity of purge was determined by subtracting the weight of the packaging film and the weight of the irradiated samples removed from the packages from the weight of the packaged sample before it was opened.

Sample pH. The pH of the samples was measured with a FC 200B pH electrode (Hanna Corporation, Hanna USA, www.hannainst.com) at 25 ºC immediately following the purge measurement by direct insertion of the electrode into the breast meat samples.

Oxidative Rancidity. Oxidative status of chicken breasts was assessed using the 2-thiobarbituric acid (TBA) distillation procedure (Tarladgis and others 1960). Absorbance of the chromophore produced by the reaction between 2-thiobarbituric acid and malonaldehyde (one of the lipid oxidation products) at 532 nm was automatically converted to mg of malonaldehyde per kg of sample by a computerized Beckman Du 640

spectrophotometer (Beckman Coulter, Canada). Duplicate TBA values per sample were measured and recorded.

Sensory evaluation. Sensory evaluation of raw and cooked chicken breasts was conducted using a trained sensory panel of faculty, staff, and students at Iowa State University. All panelists were volunteers and the project was approved by the Iowa State University Human Subjects Review Committee. A ten-member panel was utilized for raw chicken breasts, and an eight-member panel for cooked breast meat. Panelists were trained to evaluate the sensory attributes in two one-hour training sessions. Each panelist evaluated six samples per session. Three sessions were conducted each for raw and cooked chicken breast meat. A computerized sensory scoring system (COMPUSENSE five, v 4.4, Compusense, Inc. Guelph, Ontario, Canada N1H3N4) was used to collect sensory evaluation data.

Sensory evaluation for raw chicken breast meat. Individually packaged (in vacuum or MAP) chicken breasts labeled with random three-digit codes, were presented to panelists on trays that had been pre-cooled. The chicken breasts were presented cold, directly from the refrigerator (4ºC). Panelists were instructed to cut open the bag as close to the sample as possible, wait 3-5 seconds, and smell the sample. Each panelist evaluated six samples per session and three sessions (one replicate per session) were conducted. All samples were presented simultaneously and panelists were instructed to evaluate the samples in the randomized order presented on the computer screen. Testing was conducted in partitioned booths and under red fluorescent lights. Samples were evaluated for off-aroma (irradiated), sour-like aroma, and raw chicken aroma. A line

scale with 15 numerical units was used with descriptive anchors (left anchor-none, right anchor-intense) at each end of the line to evaluate each attribute of irradiated chicken breast.

Following each of the aroma sessions, the panelists evaluated the color of a single chicken breast from each treatment. The refrigerated chicken breasts, on white ceramic plates and in their original packages, were placed on a white paper background. The packages were labeled with random three digit codes. The chicken breasts were evaluated under white florescent lighting positioned to provide 70 foot-candles at the counter surface. Placement order was randomized for each session. An unstructured line scale with 15 numerical units was used to collect the data. Panelists evaluated the intensity of pink color. The left descriptor anchor was labeled none and the right anchor was labeled intense.

Sensory evaluation for cooked chicken breast meat. The chicken breasts were grilled on a George Foreman Indoor/Outdoor Grill (Model GGR62, Lake Forest, IL) to an internal temperature of 77 ºC. The temperature of the samples was monitored using a thermocouple (Chromega/Alomega) attached to an Omega digital thermometer (Model DSS-650, Omega Engineering). Three chicken breasts per treatment were prepared and panelists received two 15 mm (length) x 15 mm (width) pieces in a covered, 4-ounce Styrofoam container labeled with a random three-digit code. Care was taken so that the two pieces were from each of two of the three cooked chicken breasts. Samples were served immediately after cutting. Each panelist evaluated six samples per session and three test sessions (one replication per session) were conducted. Cooking/serving orders were randomized over the three test sessions with samples were presented sequentially in each session. Testing was conducted in partitioned booths and under red fluorescent

lights. Water and unsalted crackers were available to the panelists. A line scale (numerical value of 15 units), with descriptors representing low intensity (none) at the left and high intensity (intense) at the right, was used for scoring the following attributes: irradiated off-aroma, sour-like aroma, chicken aroma, irradiated off-flavor, sourness, and chicken flavor. Firmness (left anchor-not firm, right anchor-very firm) and juiciness (left anchor-not juicy, right anchor-very juicy) were also evaluated.

Statistical Analysis. A general linear model (SPSS 14.0 Window Grad Pack) was used to evaluate the effects of irradiation dose, packaging types and storage time. When there were significant effects or interactions ($p<0.05$) between experimental factors, linear contrast test, independent sample T-test or post-hoc tests of differences with Tukey adjustment were used to analysis the significance of main and simple main effects, or simple-simple main effects.

A mixed linear model was fit with PROC MIXED (SAS Inst., Inc., Cary, N.C., U.S.A, version 9.1) to determine the effects of irradiation dose and packaging technique on the sensory attributes. A random subject term was fitted to incorporate subject-to-subject variability. When a fixed effect was significant ($p<0.05$), post-hoc tests of differences were calculated and then adjusted with the Tukey procedure.

RESULTS AND DISCUSSION

Radiation D_{10}-value. Tables 4 and 5 present the radiation D_{10}-values of *Salmonella enterica* Typhimurium and *Campylobacter jejuni* on chicken breast meat irradiated in vacuum or high CO_2 MAP + CO packages. *Campylobacter* was more

sensitive to irradiation than *Salmonella*. The D_{10}-values in vacuum and MAP packaging were 0.31 ± 0.01 kGy and 0.29 ± 0.03 kGy , respectively, for *Campylobacter jejuni*, and 0.55 ± 0.03 kGy and 0.54 ± 0.03 kGy , respectively, for *Salmonella enterica* Typhimurium. The packaging techniques (vacuum or MAP) did not affect the D_{10}-value of these two pathogens (p-value 0.775 for *Salmonella*, and 0.587 for *Campylobacter*).

Chiasson et al. *(13)* also reported that the radiation sensitivity of *Salmonella* Typhimium in ground beef was similar in vacuum and in 100% CO_2 MAP packages, although the reported D_{10}-values (0.43 ± 0.01 kGy in vacuum, 0.42 ± 0.00 kGy in 100% CO_2 MAP) were less than in the present study. These authors reported that *Salmonella* Typhimurium in ground beef was more sensitive to irradiation when packaged in vacuum or 100% CO_2 MAP than when packaged aerobically. However, in a previous study by Chiasson et al. *(12)*, *Salmonella* Typhimurium on ground beef showed greater sensitivity to irradiation in MAP containing oxygen (60% O_2 /30% CO_2 /10% N_2) than in vacuum packaging. Patterson *(64)* reported that *Salmonella* Typhimurium in minced chicken meat was equally sensitive to irradiation in 100% CO_2 MAP or in air (0.442 kGy and 0.436 kGy, respectively). Thayer & Boyd *(81)* reported that *Salmonella* Typhimurium ATCC 14028 (inoculated in mechanically deboned chicken meat or on the surface of chicken legs) had the same radiation sensitivity in vacuum or in air when the products were treated at the same temperature. Increasing the temperature increased the lethal effect of irradiation on this bacterium. However, the same authors *(82)* also observed that if sterilized mechanically deboned meat was inoculated with *Salmonella* Typhimurium (without background microflora), this pathogen was more sensitive to irradiation in air than in vacuum. Many other studies have shown that the radiation sensitivity observed for *Salmonella* was dependent on the type of meat product *(79)*, the product temperature *(14)*,

the physiological stage of bacteria *(10)*, and the inherent radiation resistance of different serovars *(69)*.

The radiation sensitivity of *Campylobacter jejuni* was less in the present study than that reported in previous studies. Lambert & Maxcy *(41)* reported that the radiation D_{10}-value of *Campylobacter jejuni* was 0.16 kGy at 0-5 °C, in vacuum packaged ground beef, and 0.19 kGy in vacuum packaged ground turkey meat, when the temperature of products was 0-5 °C. These authors also observed that decreasing the product temperature increased the radiation resistance of this bacterium. Tarkowski et al. *(79)* reported that the radiation sensitivity of *Campylobacter* spp was product dependent. The D_{10}-values of *Campylobacter jejuni* was 0.09-0.11 kGy in filet americain, and 0.14-0.16 kGy in beef at refrigeration temperature when the product was packaged aerobically. Clavero et al. *(14)* observed that the radiation sensitivity of *Campylobacter jejuni* was lower in frozen than in refrigerated product (ground beef), and that the irradiation resistance of *Campylobacter jejuni* was affected by the fat content of the meat product. The microorganism was more resistant in low-fat ground beef when the product was irradiated at frozen temperature. Collins et al. *(15)* reported similar D_{10}-value (0.19 kGy) for *Campylobacter jejuni* in vacuum-packaged ground pork as reported for vacuum-packaged ground beef *(41)*. Patterson *(65)* observed that the radiation sensitivity of *Campylobacter* spp was strain and species dependent and reported that the D_{10}-value of *Campylobacter* spp in vacuum packaged sterilized minced chicken meat ranged from 0.12 to 0.25 kGy. Alter and Scherer *(6)* also suggested that different strains of *Campylobacter* reacted differently to environmental stressors. Therefore, a mathematical model is needed to predict the radiation sensitivity of this organism with consideration of

different strains and species in different environmental circumstances, especially for those strains directly related to human foodborne diseases.

Recovery of *Salmonella* and *Campylobacter*. There was no packaging or time effects on the recovery of *Salmonella* Typhimurium at refrigeration temperature for 24 or 48 hours (p-value 0.454 for the packaging effect, and 0.159 for the time effect; other ANOVA results not shown). *Salmonella* in irradiated or non-irradiated chicken breast meat packaged in vacuum or MAP packaging showed no significant growth in any of samples at 2-4 °C within 24 or 48 hours (data not shown).

There was no packaging or time effect on the recovery of *Campylobacter* at 2-4 °C for 24 or 48 hours (p-value 0.713 for the packaging effect, and 0.097 for the time effect; other ANOVA results not shown). Overall results showed no significant changes in the numbers of *Campylobacter* in irradiated or non-irradiated chicken breast meat in vacuum or MAP packages after 24 or 48 hours of storage (data not shown).

Salmonella is a mesophilic microorganism, and *Campylobacter* is thermophilic; therefore, it was no surprise that these organisms did not grow at refrigeration temperature, because the minimum growth temperature for *Salmonella* Typhimurium was is 6.2 °C *(35)*, and the minimum growth temperature for *Campylobacter jejuni* is 30-32 °C *(28, 36)*. However, both of these bacteria were found to be viable for a period of time. Szczawinska et al. *(77)* reported that there was no growth of several *Salmonella enterica* serotypes observed on vacuum packaged mechanically deboned chicken meat when the product was stored at 5 °C for 9 days. Dykes et al. *(18)* reported that serotypes of *Salmonella enterica* inoculated on primal beef cuts did not grow in vacuum or 100% CO_2 MAP during 2 weeks of storage at 4 °C. Dickson & Olson *(16)* reported that no

significant growth of *Salmonella enterica* serotypes in irradiated or non-irradiated ground beef was observed at 4 °C.

Campylobacter is a microaerophilic bacteria, therefore, it was expected that the numbers of this organism might be reduced if the extrinsic environment, such as packaging in vacuum or high oxygen MAP, was changed, or if storage at refrigeration temperature was introduced *(34)*. Hanninen et al. *(26)* reported that three strains of *Campylobacter jejuni* inoculated on beef decreased the numbers within 48 hours when the product was packaged in vacuum or in MAP (20% CO_2 /80% N_2, or 5% O_2 /10% CO_2 /85% N_2) and stored at 4 °C (1-2 log and 0.5-1 log, respectively). However, this effect did not occur in the present study. In the study by Stern and Kotula *(73)*, it was suggested that the diluent used for the inoculum was critical for the survival of *Campylobacter jejuni* in inoculated ground beef at 4 °C or -15 °C. Observations in the preliminary testing for our study were in accordance with what was reported by these authors. When *Campylobacter jejuni* for the present study was diluted with peptone water and inoculated on chicken breasts, the organism decreased to an undetectable level after 24 and 48 hours at 4 °C. However, when a five strain cocktail of *Campylobacter jejuni* in Muella Hinton broth was inoculated on chicken breasts, the cells remained viable through the storage time of the experiment. The number of survivors of *Campylobacter jejuni* following irradiation in the present study did not decrease significantly in chicken breast meat during refrigerated storage either in vacuum or in high CO_2 MAP. This result is consistent with the observation of Boysen et al. *(9)*, who reported that *Campylobacter jejuni* in fresh chicken fillets packaged in low oxygen MAP (70% N_2 /30% CO_2) can survive more than 10 days at 5 °C. Dykes and Moorhead *(17)* also reported that

Campylobacter jejuni can survive (without increase or decrease) in primal beef cuts packaged in vacuum or 100 % CO_2 MAP for 41 days at -1.5 °C.

The survival of *Salmonella* and *Campylobacter* during refrigerated storage. There was no significant effect of storage or packaging on the survival of *Salmonella* in irradiated or non-irradiated chicken breast meat during refrigerated storage for 6 weeks (p-value 0.140 for the storage effect, and 0.712 for the packaging effect). However, there were interactions between irradiation dose, storage period and replication (p-value: 0.000) and interaction between dose, storage and packaging (p-value: 0.000; other ANOVA results not shown). Therefore, the survival of *Salmonella* Typhimurium during the refrigerated storage is presented for each replication in tables 6 to 11.

There was a significant storage effect (p-value: 0.010), but no significant packaging effect (p-value: 0.061) on the survival of *Campylobacter* in irradiated or non-irradiated chicken breast meat packaged in vacuum or MAP. There were also interactions between irradiation dose, storage period and packaging (p-value: 0.023), and between irradiation dose, storage period and replication (p-value: 0.003; other ANOVA results not shown). The results from individual replications are presented in tables 12 to 17.

Overall results showed that there was no significant growth or reduction of *Salmonella* or *Campylobacter* in irradiated or non-irradiated chicken breast meat packaged in vacuum or MAP during the refrigerated storage. *Salmonella* in chicken breast meat packaged in vacuum showed slight growth or reduction in some of samples, such as in 1.0 kGy or 1.5 kGy in vacuum packages; however, these changes were not consistent in three replications; therefore, were most likely caused by enumeration variation. In 2.0 kGy-vacuum packages, *Salmonella* decreased significantly at the end of

storage period in two of the three replications. The survival pattern for *Salmonella* was similar in vacuum and high CO_2 MAP + CO packages, and the survivors were viable in most of the packages for 6 weeks of refrigerated storage.

Similar to *Salmonella*, *Campylobacter* survived on chicken breast meat for 6 weeks of refrigerated storage after irradiation, irrespective to irradiation dose or packaging type.

Although growth at refrigeration temperature (2-4°C) is not physiologically feasible for *Salmonella* or *Campylobacter*, many studies have shown that these two pathogens can survive on meat and poultry products through refrigerated storage *(6, 67)*. In the model developed by Eklund and Jarmund *(19)*, *Salmonella* Typhimurium did not grow but survived in air, vacuum or CO_2 packaging during 23 days of storage at 2 or 6 °C.

Campylobacter numbers in the present study remained relatively constant during 6 weeks of storage except for the reduction observed in 0.75 kGy-MAP packages after 4 to 5 weeks of storage. Blankenship and Craven *(8)* reported that the population of *Campylobacter jejuni* on raw chicken drumsticks packaged in air or in CO_2 MAP was reduced 1.5 to 2 log when the product was stored at 4 °C for 21 days. Beuchat *(7)* reported that carbon dioxide in MAP had protective function against the death of five strains of *Campylobacter jejuni* inoculated on chicken meat at 5 °C. Boysen et al. *(9)* reported that *Campylobacter* survived in anaerobic conditions longer than in air packaging or in MAP containing at least 30% O_2, therefore, these authors pointed out that the meat and poultry industry is facing a challenge when using vacuum or low oxygen MAP to extend the shelf life of products, because these conditions might compromise the food safety in regard of *Campylobacter*. This provides a very good reason for combining

food irradiation with vacuum or low MAP packaging to achieve improved food safety in the meat and poultry industry.

The growth of *Salmonella* and *Campylobacter* during temperature abuse.

After one week of post-irradiation storage at 2-4 °C, chicken breast samples were placed at room temperature (22-25 °C) for 48 hours. There was a significant temperature effect (p-value: 0.017) and irradiation dose effect (p-value: 0.012) on the growth of *Salmonella* in irradiated or non-irradiated chicken breast meat under these conditions, irrespective to packaging type (p-value: 0.141). There was also interaction between the temperature and packaging. Table 18 shows that the population of *Salmonella* Typhimurium in chicken breast meat increased significantly during temperature abuse. The cell count increased 3 logs (average) in all samples.

There was no significant temperature (p-value: 0.500) or packaging effect (p-value: 0.171) on the growth of *Campylobacter jejuni* in chicken breast meat during temperature abuse (data not shown).

The growth of *Salmonella* in either vacuum or high CO_2 MAP at room temperature (22-25 °C) in the present study was consistent with the prediction from the USDA Pathogen Modeling Program *(85)*. The parameters for obtaining a predictive growth curve were 0.5% sodium chloride content (lowest level in the program), a temperature of 25 °C, and aerobic conditions (only atmosphere in the program). The initial cell count for the model was set as 3 log /gram in the product. The predicted lag phase duration under those conditions is 2.7 hours, and 10 hours is predicted as necessary to increase the pathogen count above 3 log /gram. In the present study, there was no significant effect of CO_2 in MAP on the growth of *Salmonella* in chicken breast meat at

room temperature. Many studies have reported that the effect of CO_2 on the growth of bacteria was temperature related. Lower storage temperature has been suggested to facilitate the bacteriostatic function of CO_2 in MAP (Faber, 1991). In a study conducted by Eklund & Jarmund *(19)*, the effect of 100% CO_2 in MAP on the growth of *Salmonella* Typhimurium at 20 °C was obvious in comparison with air, vacuum or 100% N_2 MAP packaging during the first five days of incubation; however, after 14 and 23 days of incubation, the population of *Salmonella* in the CO_2 MAP exceeded the population in vacuum packages. Gill & DeLacy *(23)* observed that CO_2 MAP were significantly more effective than vacuum packaging for control of the growth of *Salmonella* Typhimurium on beef (pH 6.0) when the product was stored at 8-12 °C; however, when the storage temperature was raised to 15 °C, CO_2 lost the bacteriostatic function. Michaelson et al. *(54)* reported similar observations in their study on control of *Salmonella* in pork chops with high CO_2 MAP. *Salmonella* Typhimurium in this study did not grow at 10 °C for 35 days in high CO_2 MAP, while this organism grew in vacuum packages after 7 day of storage at the same temperature.

In the present study, there was no growth of *Campylobacter jejuni* observed at room temperature. This result agreed with previous studies which suggested that *Campylobacter* is not able to multiply when the environmental temperature is below 30 °C, although the cell remains viable even at 4 °C *(28, 31, 63, 36)*. In the present study, the population of *Campylobacter jejuni* on chicken breast meat remained constant in most of the samples at room temperature for 48 hours. This observation differs from that reported by Lee et al. *(44)*. These authors observed that *Campylobacter* was able to replicate at 4 °C or at room temperature on ground chicken or chicken skin. However, Hanninen et al. *(26)* reported that the population of *Campylobacter* on beef declined 0.5

to 1.0 log after the product was exposed to 20 °C for 24 or 48 hours. Gill and Harris *(24)* also observed a rapid decline of six strains of *Campylobacter jejuni* on sliced beef loin stored at 25 °C.

Since different strains of *Campylobacter* were used in the previous studies, and different products and packaging were used as well, it is not surprising some differences in results were observed because each strain may react to the intrinsic and extrinsic environment differently.

Color values (L* a*b*) for chicken breasts. Due to a computer malfunction, the results from one out of three replications for the color measurement of irradiated chicken breast meat were lost. For the two replications analyzed, there was no significant effect of packaging, irradiation or storage on the lightness (L*) of chicken breast meat according to ANOVA (results not shown).

The red-green color value (a*) of irradiated chicken breasts is presented in table 19. The result in replicate 1 showed that irradiation induced redness in chicken breast meat packaged in vacuum. The redness was not dose-dependent, and was persistent during 7 days of storage. The chicken breast meat packaged in high CO_2 + CO MAP was as red as irradiated vacuum packaged chicken breasts with or without irradiation due to CO, and the redness was consistent before or after the storage. However, the redness of non-irradiated chicken breasts in vacuum packages increased during 7 days storage, and the intensity of red color in all samples was similar after one week refrigerated storage, irrespective of packaging type. In replicate 2, the redness of chicken breasts in MAP was similar to replicate 1, but no significant increase of redness was induced by irradiation in chicken breast in vacuum packages. An exception to this was observed for the 1.5 kGy

vacuum packages, however, the redness in those samples faded after 7 days of storage. Irradiation and CO are both widely recognized for induced increased redness on fresh meat products. The mixed results observed in this study may be a function of the original irregular redness of chicken breasts prior to experimental treatment. Some of samples were initially as red as irradiated chicken breasts; therefore, although treated with irradiation and CO treatment, all samples were almost the same degree of redness after 7 days of storage. The initial redness of fresh meat relates to many conditions, such as the pH of the meat, the freshness of the product (storage time prior to the retail), the bulk packaging type (aerobic or anaerobic) prior to the retail packaging, freeze-thaw cycles and many other factors *(1)*.

There was no significant effect of irradiation or packaging on the yellowness of chicken breast meat.

Many studies have reported that irradiation increased the redness of pork and poultry meat (especially chicken or turkey breast meat), irrespective of the packaging atmosphere (in air, vacuum, or CO_2 MAP), and that redness was perceived as freshness of the product and preferred by sensory evaluation panels *(25, 27, 2, 60, 61)*. Nam et al. *(58)* reported that the increased red color in turkey was dependent on irradiation dose, and was persistent or increased during storage. However, observations in the present study suggested that the redness of irradiated chicken breast meat was not dose-dependent, and in some of samples the redness faded after 7 days of storage. The mechanism of forming oxymyoglobin-like pigment in irradiated fresh or cooked pork or poultry meats had been extensively studied *(22, 56, 57)*, and it was suggested that a carbon monoxide-myoglobin complex was formed in the product as a result of lower oxidation-reduction potential produced by irradiation in air or vacuum packaging. These

studies suggested that irradiation created a reduced environment in meat systems by producing a strong reducing agent such as hydrate electron from the water phase, so that the ferric iron in metmyoglobin can be reduced to ferrous iron *(22)*. If CO is produced by irradiation in meat, it is a high affinity ligand that would bind to the sixth position of heme iron to form a red pigment. This theory does not explain the brown color produced by irradiation in fresh beef, although the amount of CO presumably produced and oxidation-reduction potential would be similar in irradiated fresh beef as in irradiated pork and poultry. Therefore, it has been suggested that more factors must be involved in the color change for irradiated beef *(37)*. In the present study, 0.4% CO in MAP did not produce a red color on the surface of fresh ground beef and chicken breast meat in aerobic conditions. It appears that carbon monoxide can only bind to deoxymyoglobin that is produced by removing oxygen from heme, or that exist in anaerobic conditions. Therefore, carbon monoxide may not be as active in a meat system under atmospheric pressure as in the case of blood or muscle systems in living systems, where CO can readily replace oxygen on heme iron under the specific partial pressure *(22)*. Although Giddings & Markakis *(22)* demonstrated the oxygen replacement with CO in a solution containing radiation-generated oxymyoglobin, it is more difficult to do this with meat products. Therefore, even if irradiation can reduce metmyoglobin to form oxymyoglobin or deoxymyoglobin, it is questionable whether CO in meat products can replace oxygen on heme iron under aerobic conditions; however, this suggestion is commonly accepted to explain why irradiation can cause pinking of poultry white meat in air. Giddings & Markakis *(22)* on the other hand, suggested that irradiation reduced metmyoglobin to oxymyoglobin (red) in vacuum-packaged meat products, and oxidized metmyoglobin to ferrylmyoglobin (red) in air-packaged meat products. If this were the case for poultry and

pork, the brown color of irradiated beef packaged in air is still unexplained and further investigation is needed to provide a logical explanation.

It had been well documented that low concentrations of CO (< 1.0 %) can stabilize fresh meat color (bright cherry-red) in pork and beef. Carbon monoxide can be used to solve the problem of meat color changes caused by the long-term exposure to oxygen during display, lack of oxygen in vacuum packaging, or color changes induced by irradiation treatment *(71, 40, 39, 54)*. In the present study, 0.5% CO in high CO_2 MAP increased and/or maintained the red color of chicken breast meat, irrespective to irradiation and storage. The intensity of redness in non-irradiated or irradiated chicken breast meat packaged in high CO_2 + CO MAP was similar to irradiated chicken breasts in vacuum packages, suggesting that irradiation did not further increase the redness of chicken meat in MAP packages. However, sensory evaluation results showed that the panelists perceived a more intense redness in chicken breasts from MAP than from vacuum packages.

Package Purge and pH. Tables 20 and 21 show the pH and package purge of irradiated chicken breast meat. The pH of the chicken breast meat was not affected by irradiation, packaging or storage. There was large variation in the amount of package purge, and consequently, it was difficult to assess the effect of experimental factors. The amount of purge in fresh meat packages is, in general, directly related to pH of the product, however, in the present study, pH was not affected by treatments and averaged 5.88, which is normal for poultry products *(1)*. Previous studies have reported that high concentration CO_2 in MAP can decrease meat surface pH due to the absorption of CO_2 into the water phase of meat tissue, although the pH of whole muscle tissue was changed

little *(29, 32, 33, 72, 51)*. There also have been reports showing that high CO_2 MAP increased package purge (drip loss) and cooking loss *(68, 39, 86)*, although the pH of meat product was not changed. Although cooking loss was not measured in the present study, sensory evaluation (tables 27 and 28) showed that the cooked chicken breasts from high CO_2 + CO MAP were firmer and less juicy than chicken breasts from vacuum packages. This result is similar to the study of Michaelson et al. *(54)*, who reported that pork chops from high CO_2 + CO MAP were less tender than the chops from vacuum packages.

Oxidation rancidity. There was no significant effect of experimental treatments on lipid oxidation of irradiated chicken breast meat (ANOVA not shown). Table 22 shows that in two out of three replications, TBA values increased slightly in chicken breast meat in 1.5 kGy-MAP packages, however, the TBA values of all samples were low (less than 0.75).

One of the advantages of vacuum packaging is to eliminate oxygen from packages if meat products are treated with irradiation. Research has shown that lipid oxidation caused by irradiation can be mitigated with vacuum packaging *(47, 3)*. The result of the present study showed that high CO_2 MAP achieved the same function as vacuum and prevented lipid oxidation in meat products during irradiation treatment. This result is consistent with the observation in the study of Kusmider et al. *(40)*, who reported that the TBA values of ground beef (irradiated at 2.0 or 4.5 kGy) in vacuum packages and in MAP + CO (0.5% CO / 25.5% N_2 / 70% CO_2) packages were not significantly different after irradiation or after at least 3 days of storage. Krause et al. *(39)* also reported that the TBA values of pork chops were not significantly different in vacuum packages compared to MAP + CO packages.

Sensory evaluation. The results of the sensory evaluation for raw irradiated chicken breast meat are presented in tables 23, 24 and 25. Fresh irradiated chicken breast meat had significantly stronger irradiated off-odor, and less chicken breast aroma, irrespective of irradiation dose or packaging. The high CO_2 + CO MAP packages resulted in significantly more intensive sour-like aroma than vacuum packages. Irradiated chicken breast meat from vacuum packages (without CO) was redder than non-irradiated, and irradiated samples from high CO_2 + CO MAP were redder than irradiated samples from vacuum packages. Tables 26, 27 and 28 show the results of sensory evaluation for cooked chicken breast meat. There was no significant irradiated off-odor or off-flavor observed in cooked irradiated chicken breast meat compared to non-irradiated, although the panelists reported a slight off-flavor in chicken meat from MAP. Irradiated chicken breast meat has less chicken flavor than non-irradiated. Compared to chicken meat from vacuum packages, the samples from MAP were firmer and slightly less juicy.

Irradiated off-odor or off-flavor has been one of the major hindrances for consumer acceptance of irradiated meat product *(42)*. Many volatiles, such as hydrocarbons and sulfur compounds, produced by irradiation, have been reported to be responsible for off-odor in irradiated fresh meat *(58, 38)*. Meat from different animal species has been found to produce different radiolytic volatiles from irradiation, and packaging (air or vacuum) also affects the form and quantity of those volatiles. However, some research has suggested that the cooking process can eliminate a significantly amount of off-odor or off-flavor from irradiated chicken meat *(27, 2)*. In the present study, panelists could not detect irradiated off-odor or off-flavor from cooked irradiated chicken

breast meat packaged in either vacuum or MAP packages. However, sour-like odor was observed for raw chicken breasts from high CO_2 MAP packages.

CONCLUSIONS

Irradiation was effective for eliminating *Salmonella* Typhimurium or *Campylobacter jejuni* in fresh chicken breast meat. With the doses of 1.5 kGy and 0.75 kGy, irradiation reduced 2.8 log of *Salmonella* and 2.5 log of *Campylobacter* in chicken breasts, respectively, regardless of vacuum or high CO_2 MAP. Therefore, if these packaging techniques are applied for extension shelf life of poultry products, irradiation is a feasible means for improving control of the pathogens.

The results from microbiological assessments and quality evaluation in this study indicated that high CO_2 MAP did not demonstrated any advantage over vacuum for improved control of *Salmonella* Typhimurium and *Campylobacter* jejuni on chicken breast meat. The survivors of both foodborne pathogens did not grow, but persistent in vacuum or high CO_2 + CO MAP through 6 weeks of refrigerated storage. Further, *Salmonella* increased in number on chicken breasts when the product was exposed to room temperature for 48 hours. Therefore, if the initial contamination of these pathogens is high, undercooked breast meat, cross contamination with RTE food or temperature abuse of the product may still be a food safety concern, regardless of irradiation treatment with doses evaluated in this study.

Despite the bacteriocidal function of irradiation, further study is needed to mitigate potential irradiated off-odor in fresh chicken breast meat to gain more consumer acceptance for irradiated meat products. Because irradiation increased red color of chicken breasts, is not necessary to use CO in high CO_2 MAP. A comprehensive

prediction model is also needed for the survival and growth of these foodborne pathogens under a variety of different controlled conditions, so that the industry can improve the control measures by utilizing risk-based information and modeling predictions.

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Table 1--Absorbed irradiation doses by chicken breast meat (for *Salmonella*) packaged in vacuum and high CO_2 MAP + CO (experiment 1)

Target doses (kGy)	Average surface dose (kGy)	Average maximum dose (kGy)	Overall average dose (kGy)
0.5 kGy	0.503 kGy	0.675 kGy	0.593 kGy
1.0 kGy	1.013kGy	1.414 kGy	1.216 kGy
1.5 kGy	1.533 kGy	2.059 kGy	1.781 kGy

Table 2--Absorbed irradiation doses by chicken breast meat (for *Campylobacter*) packaged in vacuum and high CO_2 MAP + CO (experiment 1)

Target doses (kGy)	Average surface dose (kGy)	Average maximum dose (kGy)	Overall average dose (kGy)
0.25 kGy	0.267 kGy	0.347 kGy	0.314 kGy
0.5 kGy	0.257 kGy	0.722 kGy	0.600 kGy
0.75 kGy	0.742 kGy	1.001 kGy	0.871 kGy

Table 3--Absorbed irradiation doses by chicken breast meat packaged in vacuum and high CO_2 MAP + CO (experiment 2)

Target doses (kGy)	Average surface dose (kGy)	Average maximum dose (kGy)	Overall average dose (kGy)
1.0 kGy	1.013 kGy	1.430 kGy	1.213 kGy
1.5 kGy	1.523 kGy	1.970 kGy	1.746 kGy

Table 4—Mean of radiation D_{10}-values (kGy) of *Salmonella* Typhimurium in chicken breast meat packaged in vacuum or in high CO_2 MAP + CO

Packaging	N	Mean D_{10}-value	Std. Deviation	Std. Error Mean	p-value
Vacuum	9	0.55	0.07	0.03	0.775
MAP	9	0.54	0.08	0.03	

Table 5—Mean of radiation D_{10}-values (kGy) of *C. jejuni* in chicken breast meat packaged in vacuum or in high CO_2 MAP + CO

Packaging	N	Mean D_{10}-value	Std. Deviation	Std. Error Mean	p-value
Vacuum	9	0.31	0.03	0.01	0.587
MAP	9	0.29	0.08	0.03	

Table 6—The survival of *Salmonella* Typhimurium (log cfu /g) on irradiated chicken breast meat packaged in vacuum during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.20[a,b]	0.10	5.00[a,b]	0.10	4.96[a,b]	0.08	5.48[b]	0.25	5.15[a,b]	0.16	4.58[a]	0.17
0.5	4.40[a]	0.15	4.92[a]	0.91	4.46[a]	0.45	4.54[a]	0.08	2.10[b]	0.11	4.14[a,b]	0.32
1.0	2.81[a,b]	0.18	2.51[a,b]	0.11	2.76[a,b]	0.27	2.09[a]	0.24	3.59[b]	0.37	3.45[b]	0.09
1.5	2.61	0.42	1.14	0.48	1.12	0.49	1.08	1.06	1.16	0.78	0.58	0.19

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 7— The survival of *Salmonella* Typhimurium (log cfu /g) on irradiated chicken breast meat packaged in vacuum during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	$5.22^{a,b}$	0.14	$5.15^{a,b}$	0.17	$4.91^{a,b,c}$	0.11	$4.70^{b,c}$	0.25	4.21^{c}	0.14	$4.48^{a,b,c}$	0.08
0.5	3.82^{a}	0.08	3.91^{a}	0.33	2.66^{b}	0.17	$3.30^{a,b}$	0.27	$3.53^{a,b}$	0.27	$3.71^{a,b}$	0.12
1.0	2.61	0.10	2.60	0.32	2.14	0.25	2.03	0.18	1.89	0.12	1.75	0.15
1.5	$1.78^{a,b}$	0.26	2.27^{a}	0.16	$2.00^{a,b}$	0.20	$1.27^{b,c}$	0.20	$0.85^{c,d}$	0.00	0.16^{d}	0.00

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 8— The survival of *Salmonella* Typhimurium (log cfu /g) on irradiated chicken breast meat packaged in vacuum during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.02	0.17	6.39	1.00	5.46	0.22	5.24	0.13	5.23	0.38	5.03	0.16
0.5	$3.95^{a,b,c}$	0.28	4.53^{b}	0.12	$4.44^{a,b}$	0.27	3.13^{c}	0.22	$3.83^{a,b,c}$	0.32	3.31^{c}	0.12
1.0	2.98	0.44	3.08	0.25	2.86	0.16	2.40	0.24	2.28	0.30	2.59	0.22
1.5	1.81^{a}	0.25	1.74^{a}	0.19	$1.25^{a,b}$	0.57	$1.10^{a,b}$	0.10	$1.08^{a,b}$	0.39	0.16^{b}	0.16

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 9—The survival of *Salmonella* Typhimurium (log cfu /g) on irradiated chicken breast meat packaged in high CO_2 MAP + CO during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.53^{a}	0.08	$4.09^{b,c}$	0.43	3.75^{c}	0.10	$4.61^{a,b,c}$	0.06	$4.51^{b,c}$	0.14	$5.02^{a,b}$	0.09
0.5	4.45^{a}	0.27	4.60^{a}	0.12	4.59^{a}	0.05	3.91^{a}	0.15	2.43^{b}	0.38	4.33^{a}	0.28
1.0	$3.68^{a,b}$	0.12	$3.03^{a,b}$	0.84	2.20^{a}	0.13	$3.37^{a,b}$	0.26	4.54^{b}	0.13	$3.37^{a,b}$	0.31
1.5	1.48	0.32	1.42	0.45	1.45	0.43	0.49	0.75	1.10	0.09	1.42	0.17

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 10— The survival of *Salmonella* Typhimurium (log cfu /g) on irradiated chicken breast meat packaged in high CO_2 MAP + CO during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.32[a,b]	0.07	5.56[b]	0.05	5.30[a,b]	0.17	5.27[a,b]	0.10	5.00[a]	0.16	4.97[a]	0.06
0.5	4.46	0.10	4.04	0.45	4.28	0.21	3.74	0.20	3.76	0.16	3.65	0.18
1.0	3.54[a]	0.18	3.50[a]	0.14	3.25[a,b]	0.05	3.22[a.b,c]	0.08	2.88[b]	0.02	2.76[c]	0.03
1.5	2.27[a]	0.24	1.81[a]	0.27	2.69[a]	0.26	1.74[a]	0.21	0.36[b]	0.18	0.33[b]	0.20

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 11—The survival of *Salmonella* Typhimurium (log cfu /g) on irradiated chicken breast meat packaged in high CO_2 MAP + CO during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	4.75	0.24	5.22	0.13	5.34	0.08	5.18	0.24	5.23	0.23	5.52	0.10
0.5	3.97[a,b]	0.27	3.91[a,b]	0.16	4.25[a]	0.18	3.21[b]	0.20	4.53[a]	0.18	3.28[c]	0.07
1.0	3.16	0.08	3.19	0.15	2.80	0.24	2.76	0.14	2.68	0.10	3.05	0.27
1.5	1.80	0.21	2.30	0.09	2.42	0.12	1.77	0.46	1.98	0.32	2.00	0.25

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 12—The survival of *C. jejuni* (log cfu /g) on irradiated chicken breast meat packaged in vacuum during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	6.14	0.08	6.17	0.13	6.09	0.15	6.24	0.29	6.04	0.16	5.83	0.09
0.25	5.35	0.05	5.45	0.09	5.14	0.03	5.35	0.10	5.08	0.05	4.80	0.35
0.50	3.67^{a}	0.27	$4.20^{a,b}$	0.20	$4.51^{a,b}$	0.04	4.58^{b}	0.29	$3.73^{a,b}$	0.04	$3.87^{a,b}$	0.07
0.75	$3.72^{a,b}$	0.05	3.71^{a}	0.08	3.81^{a}	0.12	$3.52^{a,b}$	0.12	3.08^{b}	0.27	2.22^{c}	0.07

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 13— The survival of *C. jejuni* (log cfu /g) on irradiated chicken breast meat packaged in vacuum during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	6.00	0.06	6.11	0.14	6.09	0.05	5.97	0.09	6.01	0.03	5.99	0.15
0.25	5.15	0.03	4.91	0.08	5.31	0.11	5.44	0.05	5.45	0.26	4.97	0.04
0.50	4.05	0.06	3.91	0.15	3.64	0.08	3.59	0.27	3.61	0.06	3.91	0.27
0.75	3.60	0.12	3.60	0.08	3.61	0.29	3.46	0.24	3.10	0.17	3.40	0.10

[1]No significant difference between the means within the each row ($p < 0.05$)
[2]Standard error of means
[3]The target irradiation dose

Table 14— The survival of *C. jejuni* (log cfu /g) on irradiated chicken breast meat packaged in vacuum during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.97[a,b,c]	0.07	6.50[b]	0.15	5.51[a,c]	0.13	5.81[a,b,c]	0.07	5.35[c]	0.30	5.97[a,b,c]	0.01
0.25	5.11[a]	0.15	4.87[a,b]	0.16	4.84[a,b]	0.03	4.56[a,b]	0.24	4.28[b]	0.13	4.48[a,b]	0.22
0.50	4.05	0.06	3.91	0.15	3.64	0.08	3.59	0.27	3.61	0.06	3.91	0.27
0.75	3.17	0.02	2.66	0.56	2.80	0.08	2.61	0.05	2.68	0.21	2.37	0.14

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 15—The survival of *C. jejuni* (log cfu /g) on irradiated chicken breast meat packaged in high CO_2 MAP + CO during refrigerated storage (Rep 1)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	6.04	0.03	6.24	0.09	6.07	0.16	6.00	0.10	6.11	0.09	5.77	0.04
0.25	5.09	0.23	5.28	0.07	5.06	0.10	5.43	0.00	5.02	0.07	4.95	0.15
0.50	3.90	0.12	3.70	0.17	3.44	0.12	3.63	0.07	3.27	0.10	3.29	0.22
0.75	$3.50^{a,b}$	0.24	3.58^{a}	0.22	3.66^{a}	0.03	$2.82^{b,c}$	0.14	2.15^{c}	0.12	1.62^{d}	0.07

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 16— The survival of *C. jejuni* (log cfu /g) on irradiated chicken breast meat packaged in high CO_2 MAP + CO during refrigerated storage (Rep 2)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	6.01[a]	0.05	5.86[a,b]	0.04	5.71[a,b]	0.07	6.09[a]	0.20	5.30[b]	0.22	5.82[a,b]	0.05
0.25	5.09[a,b]	0.03	4.90[a,b]	0.15	5.52[a]	0.17	5.42[a]	0.07	5.21[a,b]	0.16	4.68[b]	0.16
0.50	4.89[a]	0.66	3.35[b]	0.22	4.50[a,b]	0.11	3.74[a.b]	0.06	3.56[a,b]	0.06	3.70[a,b]	0.11
0.75	3.67[a]	0.17	2.86[a,b,c]	0.25	3.29[a,b]	0.27	2.71[b,c]	0.16	2.49[c]	0.05	2.24[c]	0.05

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 17—The survival of *C. jejuni* (log cfu /g) on irradiated chicken breast meat packaged in high CO_2 MAP + CO during refrigerated storage (Rep 3)

Dose[3] (kGy)	Storage Period (week)											
	1		2		3		4		5		6	
	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]	Mean[1] (log /g)	SE[2]
0	5.90	0.05	6.78	1.08	5.26	0.06	5.67	0.23	4.97	0.25	5.55	0.10
0.25	5.04[a]	0.04	4.72[a]	0.16	4.96[a]	0.04	4.70[a]	0.10	3.73[b]	0.09	4.81[a]	0.16
0.50	3.90	0.12	3.70	0.17	3.44	0.12	3.63	0.07	3.27	0.10	3.29	0.22
0.75	2.83	0.27	2.29	0.22	2.39	0.10	2.09	0.30	1.84	0.20	1.87	0.09

[1]Mean values within same row with different superscripts are significantly different ($p < 0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 18—The growth of *Salmonella* Typhimurium (log cfu /g) on irradiated chicken breast meat at 25ºC for 48 hours

Dose[3] (kGy)	Count (log cfu /g) in Vacuum packages				Count (log cfu /g) in MAP packages			
	Mean[1] (4 ºC)	SE[2]	Mean[1] (25ºC)	SE[2]	Mean[1] (4 ºC)	SE[2]	Mean[1] (25ºC)	SE[2]
0	5.52[a]	0.34	8.04[b]	0.13	4.96[a]	0.13	7.94[b]	0.53
0.5	4.45[a]	0.32	7.31[b]	0.36	4.18[a]	0.15	6.93[b]	0.44
1.0	2.73[a]	0.18	7.21[b]	0.14	2.97[a]	0.08	5.26[b]	0.49
1.5	1.71[a]	0.27	6.46[b]	0.08	1.84[a]	0.22	5.66[b]	0.01

[1]Mean values within the same row of the same packaging type with different superscripts are significantly different ($p<0.05$).
[2]Standard error of means
[3]The target irradiation dose

Table 19—The red-green color value (a*) of chicken breast meat irradiated in vacuum and high CO_2 MAP + CO packages

Dose[3] (kGy)	[1,2] Replication 1				[1,2] Replication 2			
	Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	10.81[a] ± 0.31	13.08[a,b] ± 0.85	13.77[b] ± 0.50	15.04[b] ± 0.52	12.03[a] ± 0.53	12.44[a] ± 0.42	13.88[a] ± 0.86	14.53[a] ± 0.50
1.0	14.83[b] ± 0.71	13.14[b] ± 0.24	14.50[b] ± 0.25	14.18[b] ± 0.36	13.08[a] ± 0.40	13.80[a] ± 0.60	13.22[a] ± 0.49	12.80[a] ± 0.28
1.5	14.14[b] ± 0.72	13.85[b] ± 0.36	13.99[b] ± 0.59	14.49[b] ± 0.68	16.17[b] ± 0.69	13.87[a,b] ± 0.87	14.36[a,b] ± 0.54	14.21[a,b] ± 0.44

[1] Mean values within same row and same column in the same application with different superscripts are significantly different ($p < 0.05$).
[2] Standard error of means
[3] The target irradiation dose

Table 20—The pH of chicken breast meat irradiated in vacuum and high CO_2 MAP + CO packages

Dose[3] (kGy)	Vacuum				MAP			
	Day 1		Day 7		Day 1		Day 7	
	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]
0	5.91	0.03	5.76	0.00	5.79	0.02	5.84	0.02
1.0	5.88	0.04	5.84	0.01	6.36	0.57	5.83	0.05
1.5	5.83	0.02	5.91	0.04	5.85	0.02	5.80	0.02

[1] No significant difference between the means within same row and same column ($p < 0.05$)
[2] Standard error of means
[3] The target irradiation dose

Table 21—Purge (grams) of chicken breast meat irradiated in vacuum and high CO_2 MAP + CO packages

Dose[3] (kGy)	Vacuum				MAP			
	Day 1		Day 7		Day 1		Day 7	
	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]	Mean[1]	SE[2]
0	3.57[a,b]	0.07	5.53[a]	0.41	2.15[b]	1.46	2.91[b]	1.06
1.0	2.93[a,b]	0.75	4.20[a]	0.20	1.58[b]	0.15	2.92[a,b]	1.26
1.5	1.71[a]	0.01	3.08[a]	0.75	1.64[a,b]	0.13	2.80[a]	0.35

[1] Mean values within same row and same column with different superscripts are significantly different ($p < 0.05$).
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 22—The TBA values of chicken breast meat irradiated in vacuum and high CO_2 MAP + CO packages

Dose[3] (kGy)	[1,2] Replication 1				[1,2] Replication 2				[1,2] Replication 3			
	Vacuum		MAP		Vacuum		MAP		Vacuum		MAP	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	0.27^{a} ± 0.06	0.21^{a} ± 0.00	0.33^{a} ± 0.08	0.18^{a} ± 0.03	0.25^{a} ± 0.00	0.29^{a} ± 0.05	$0.34^{a,b}$ ± 0.01	0.34^{a} ± 0.01	0.24^{a} ± 0.00	$0.32^{a,c}$ ± 0.04	$0.25^{a,c}$ ± 0.04	$0.26^{a,c}$ ± 0.02
1.0	0.39^{a} ± 0.05	0.40^{a} ± 0.04	0.29^{a} ± 0.03	$0.45^{a,c}$ ± 0.07	0.25^{a} ± 0.01	0.28^{a} ± 0.02	0.50^{b} ± 0.13	0.24^{a} ± 0.00	$0.22^{a,b}$ ± 0.00	0.17^{b} ± 0.00	$0.34^{a,c}$ ± 0.05	$0.33^{a,c}$ ± 0.05
1.5	$0.37^{a,c}$ ± 0.06	$0.37^{a,c}$ ± 0.04	0.72^{b} ± 0.12	0.65^{c} ± 0.01	$0.28^{a,b}$ ± 0.02	0.24^{a} ± 0.03	0.47^{b} ± 0.03	$0.37^{a,b}$ ± 0.00	0.37^{a} ± 0.04	0.20^{c} ± 0.01	0.20^{c} ± 0.01	0.20^{c} ± 0.00

[1]Mean values within same row and same column with different superscripts are significantly different ($p < 0.05$) within the same replication.
[2] Each means ± standard error of means
[3]The target irradiation dose

Table 23—LS means[1,2] for sensory attributes of raw chicken breasts packaged using different techniques

Packaging	Off-Aroma Irradiated[4]	Sour-Like Aroma[4]	Raw Chicken Aroma[4]
Vacuum	4.0	1.3[a]	2.6
MAP[3]	4.5	2.4 [b]	2.4
SEM[5]	0.6	0.6	0.4

[1]Data for irradiation treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2] Means in a column followed by a different letter are significantly different ($p<0.05$).
[3]Modified atmosphere packaging.
[4]Line scale, numerical value of 15; none=0, intense =15.

Table 24—LS means[1,2] for sensory attributes of raw chicken breast irradiated at different levels

Dose (kGy)	Off-Aroma[4] Irradiated	Sour-Like Aroma[3]	Raw Chicken Aroma[3]
0	0.9 [a]	1.0 [a]	4.0 [a]
1.0	5.4 [b]	2.3 [b]	2.0 [b]
1.5	6.5 [b]	2.2 [b]	1.6 [b]
SEM[4]	0.6	0.6	0.4

[1]Data for packaging treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2]Means in a column followed by a different letter are significantly different ($p<0.05$).
[3]Line scale, numerical value of 15; none=0; intense=15.
[4]± standard error of the mean

Table 25—LS means[1,2] for sensory attributes of raw chicken breasts packaged using different techniques and irradiated at different levels

Treatment (kGy)	Pink[4]
Vacuum, 0	2.8 [a]
Vacuum, 1.0	6.4 [b]
Vacuum, 1.5	7.7 [bc]
MAP[3], 0	7.8 [bc]
MAP, 1.0	13.4 [d]
MAP, 1.5	8.9 [c]
SEM[5]	0.5

[1]An interaction was noted between the packaging and irradiation treatments. Individual treatment means are, therefore, reported.
[2]Means in a column followed by a different letters are significantly different ($p<0.05$).
[3]Modified atmosphere packaging
[4]Line scale, numerical value of 15; none=0; intense=15.
[5]± standard error of the mean

Table 26—LS means [1,2] for sensory attributes of cooked chicken breasts packaged using different techniques

Packaging	Off-Aroma Irradiated[4]	Sour-Like Aroma[4]	Chicken Aroma[4]	Firmness[5]	Off-Flavor Irradiated[4]	Sourness[4]	Chicken Flavor[4]
Vacuum	2.3	0.8	3.5	5.6 [a]	1.9 [a]	1.5	3.8
MAP[3]	2.6	0.7	3.1	6.7 [b]	3.0 [b]	2.0	3.1
SEM[6]	0.5	0.3	0.4	0.7	0.4	0.4	0.6

[1]Data for irradiation treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2]Means in a column followed by a different letter are significantly different ($p<0.05$).
[3]Modified atmosphere packaging
[4]Line scale, numerical value of 15; none=0; intense =15.
[5]Line scale, numerical value of 15; not firm=0; very firm=15.
[6]± standard error of the mean.

Table 27—LS means[1,2] ± standard errors for sensory attributes of cooked chicken breasts irradiated at different levels

Dose (kGy)	Off-Aroma Irradiated[3]	Sour-Like Aroma[3]	Chicken Aroma[3]	Firmness[4]	Off-Flavor Irradiated[3]	Sourness[3]	Chicken Flavor[3]
0	2.3	0.7	3.7	5.3 [a]	1.7	1.9	4.1[a]
1.0	2.6	0.8	3.4	6.7 [b]	2.9	1.6	3.3[ab]
1.5	2.5	0.9	2.9	6.6 [b]	2.7	1.8	2.9[b]
SEM[5]	0.5	0.3	0.4	0.7	0.5	0.4	0.6

[1]Data for packaging treatments were pooled since no interaction between packaging and irradiation effects was observed.
[2]Means in a column followed by a different letter are significantly different ($p<0.05$).
[3]Line scale, numerical value of 15; none=0; intense =15.
[4]Line scale, numerical value of 15; not firm=0; very firm=15.
[5]± standard error of the mean.

Table 28—LS means[1,2] for sensory attributes of cooked chicken breast packaged using different techniques and irradiated at different levels

Treatment (kGy)	Juiciness[4]
Vacuum, 0	6.1 [bc]
Vacuum, 1.0	7.7 [c]
Vacuum, 1.5	6.1 [bc]
MAP[3], 0	5.6 [ab]
MAP, 1.0	3.8 [a]
MAP, 1.5	5.2 [ab]
SEM[5]	0.6

[1]An interaction was noted between the packaging and irradiation treatments. Individual treatment means are, therefore, reported.
[2]Means in a column followed by a different letter are significantly different ($p<0.05$).
[3]Modified atmosphere packaging.
[4]Line scale, numerical value of 15; not juicy=0, very juicy=15.
[5]± standard error of the mean.

GENERAL CONCLUSION

The data from in this study has resulted in several conclusions. First, irradiation can effectively reduce *Escherichia coli O157:H7* in ground beef patties, *Listeria monocytogenes* in frankfurters and pre-cooked pork chops, *Salmonella enterica* Typhimurium and *Campylobacter jejuni* in fresh chicken breast meat when these products were packaged in either vacuum or high CO_2 modified atmosphere packaging (MAP). Radiation sensitivities of these four foodborne pathogens on the respective products were similar with both packaging methods. Second, high CO_2 MAP can inhibit the growth of *L. monocytogenes* on ready-to-eat (RTE) meat products during refrigerated storage for a longer period of time in comparison to vacuum packaging. Third, neither vacuum packaging, nor high CO_2 MAP can further eliminate survivors of these pathogens during refrigerated storage or under temperature abuse at room temperature. Therefore, if irradiation cannot completely inactivate these organisms, they are likely to survive during refrigerated storage (6 week for fresh meat, 12 weeks for RTE meat) or, the organisms may even grow if the products are exposed to room temperature for a specific time. Fourth, high CO_2 MAP + CO stabilizes bright cherry-red ground beef color when ground beef is treated with irradiation for safety. Similar to vacuum packaging, high CO_2 MAP can also prevent lipid oxidation in meat products that may be induced by irradiation. However, a high concentration of CO_2 in MAP may cause texture changes in some meat products, such as in frankfurters, as a result of excessive CO_2 absorption, producing pores during heating. Meat products packaged in high CO_2 tend to have stronger sour-like aroma comparing to vacuum packaged meat products. Last, irradiated off-odor in meat products packaged with either vacuum or high CO_2 MAP remains a

problem to be overcome, so that the consumer acceptance of irradiated meat products can be improved.

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